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TITLE: Caffeine, Adenosine Receptors and Estrogen in Toxin Models of Parkinson's Disease

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14. ABSTRACT: Continued progress has been made toward each of the 3 Specific Aims (SAs) of our research project, "Caffeine, adenosine receptors and estrogen in toxin models of Parkinson's disease (PD)". The overarching hypothesis of the project is that multiple environmental protectants and toxins interact to influence the health of the dopaminergic neurons lost in PD. To that end we are characterizing the interplay between several environmental agents (pesticides, caffeine and estrogen) that are leading candidate modulators of PD risk. Main accomplishments during the Year 3 of the project: 1) Demonstration that neuronal forebrain A2A receptors can play a critical role in dopaminergic neuron injury in the MPTP model of neurodegeneration in Parkinson's disease. 2) Using a powerful newly Cre/LoxP conditional knockout system, we have obtained evidence that it is the neuronal forebrain A2A receptors in the striatum that are responsible for this toxicity. Thus it is through these receptors that caffeine and more specific antagonists of the adenosine A2A receptor may offer neuroprotection against the development or progression of PD. 3) Demonstration for the first time that caffeine's neuroprotective effect extends to the dual pesticide – paraquat plus maneb – model of PD, a chronic, potentially more environmentally relevant model of the disease. 4) Demonstration for the first time that urate -- a caffeine analog and antioxidant linked to slower PD progression and risk -- can be neuroprotective in an in vivo model of PD. This finding may have a particularly rapid translational impact as urate-elevating therapy is now being pursued as potential neuroprotectant for PD patients. 5) Methodological advances were achieved with a viral vector-based Cre/LoxP conditional knockout system. It will allow us to dissect caffeine and A2A receptor involvement in neurotoxin models of PD with an unprecedented combination of anatomical and molecular precision in different brain structures.					
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Introduction

Identifying the mechanisms by which caffeine and more specific A_{2A} antagonists protect dopaminergic neurons in multiple toxin models of Parkinson's disease (PD) will advance our knowledge of the pathophysiology, epidemiology and therapeutics of PD.

The *overarching hypothesis* pursued by this proposal is that **multiple environmental protectants and toxins interact to influence of the health of the dopaminergic neurons lost in Parkinson's disease**. Here we endeavor to characterize the interplay between several environmental agents (pesticides, caffeine and estrogen) that are leading candidate modulators of PD risk.

We are pursuing 3 *specific hypotheses*:

- 1) Caffeine acts through blockade of brain A_{2A}Rs to protect dopaminergic neurons in both acute and chronic toxin models of PD. (*Specific Aim #1*)
- 2) Caffeine perfusion and focal A_{2A}R inactivation within the striatum are sufficient to attenuate MPTP toxicity, by reducing toxin-induced release of glutamate and/or GABA. (*Specific Aim #2*)
- 3) Estrogen attenuates the protective effect of caffeine but not the protective of A_{2A}R deletion because it acts by altering caffeine metabolism or A_{2A}R expression. (*Specific Aim #3*)

Statement of Relevance (from our proposal)

A. Parkinson's Disease -

Basic neuroscience significance - The results will improve our understanding of adenosine receptor neurobiology, and will provide insight into the role of endogenous adenosine in basal ganglia biology physiology and PD pathophysiology.

Epidemiological significance - Establishing the ability of caffeine to protect dopaminergic neurons in PD models and identifying a plausible mechanism of action greatly strengthens the hypothesis that a neuroprotective effect of caffeine is the basis for its inverse epidemiological association with risk of PD.

Therapeutic significance - With several specific adenosine A_{2A} antagonists emerging as promising therapeutic candidates based on their motor-enhancing (symptom-relieving) action, the prospects for additional neuroprotective benefit substantiated by this project may considerably enhance their therapeutic potential. In addition, identifying a biological basis for caffeine-estrogen interaction in modifying PD risk could also affect recommendations for estrogen replacement strategies in women with PD taking A_{2A} antagonists or caffeine (and *vice versa*). Furthermore, based on evidence that A_{2A}Rs contribute to the neurotoxicity affecting cortical and striatal neurons (as well as dopaminergic neurons), our findings may support novel A_{2A}R-

based neuroprotective treatments for a wider range of neurological diseases from stroke to amyotrophic lateral sclerosis (ALS) to Alzheimer's disease.

B. Environmental Neurotoxin Exposure in Military Service – By characterizing the neuroprotective effects of caffeine in a chronic pesticide model of PD (as well as the acute MPTP model), the proposed work will define a prototypical interaction between environmental toxins and protectants in determining the extent of a well-characterized neurological lesion (dopaminergic neuron death). Although there has been no compelling evidence to suggest that the incidence of PD will itself increase in association with military service or combat theatre exposures,^[1] putative toxin exposure in the military may be linked to the development of another debilitating neurodegenerative disorder, ALS.^[2] Moreover, some objective biological measures in veterans diagnosed with a “Persian Gulf War syndrome” have indicated dysfunction of dopaminergic neurotransmission in the basal ganglia,^[3] raising the possibility (together with other data^[4]) of altered risk for PD in this group. In any event, establishing a biological precedent for neurotoxin-neuroprotectant interplay in the relatively common disorder of PD, may provide a ‘roadmap’ that can be used should any neurological illness be confirmed to develop in association with prior military exposures.

C. Understanding the Non-stimulant CNS Effects of Caffeine. The psychoactive agent caffeine has been endorsed for military use at relatively high doses to help maintain operational readiness.^[5] This recommendation has been based on a large body of evidence demonstrating sustainment of mental task performance by caffeine, and a lack of evidence for substantial harm at these doses. However, adopting the use of any CNS-active drug by protocol warrants careful consideration of newly appreciated neuronal actions of the agent. Accordingly, the proposed investigation of the novel neuroprotective effect of caffeine and its underlying mechanisms (e.g., altered neurotransmitter release) would be of significance for military programs that provide specific doses of caffeine to personnel to enhance cognitive function.

D. Gender Differences in How Environmental Factors Impact Toxin Susceptibility. Our investigation of how caffeine and estrogen exposures interact to modify neurotoxin susceptibility in laboratory models of PD may have substantial significance for the human epidemiology that prompted our pursuit of this line of research. In addition, the proposed studies may provide a prototype for modeling how gender and estrogen status interact with environmental exposures of relevance to the military (i.e., neurotoxins, caffeine). A better appreciation of how gender alters susceptibility to environmental toxins or protectants may ultimately lead to a better understanding (and modification) of the differential risks faced by women and men serving in the same military operations.

Body of the Report

Progress during Year 3 on Specific Aims and experiments as laid out in our Statement of Work (SOW [in blue]) is described here in detail.

STATEMENT OF WORK (focus on main areas of progress in Yr 3)

Specific Aim #1 – to definitively determine whether brain A_{2A}Rs or A₁Rs contribute to dopaminergic neuron degeneration in acute and chronic toxin models of PD, and whether the brain A_{2A}R is required for caffeine's protective effect in these PD models.

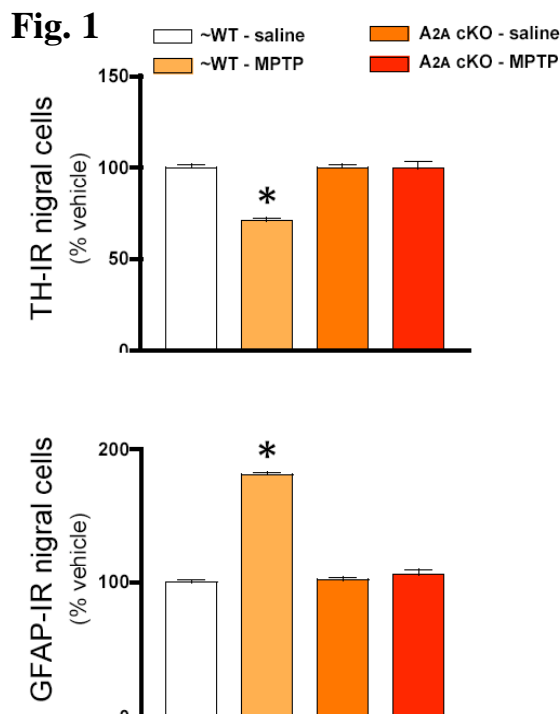
[Please see abstract publications in Appendices A and B.]

Hypothesis 1: Caffeine acts through blockade of brain A_{2A} (not A₁) receptors to protect dopaminergic neurons in both acute (MPTP) and chronic (paraquat/maneb) toxin models of PD.

Exp# 2 – Effect of brain-specific A_{2A} KO in MPTP and Pq/Mb models.

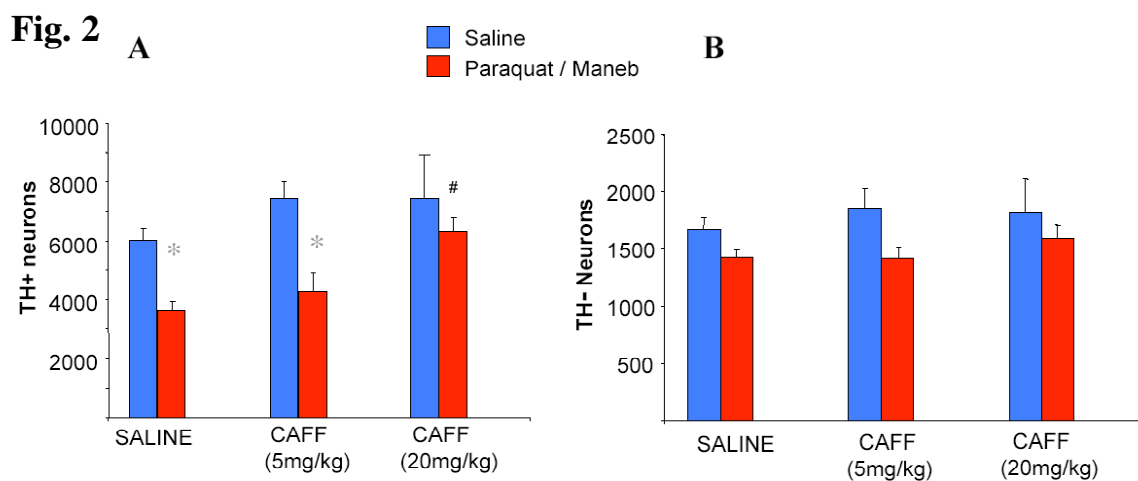
As reported for Year 1, we completed the generation and initial characterization of a conditional (Cre/loxP system) KO of post-natal forebrain neuronal A_{2A} receptors. The *CamKIIα* promoter was used to drive expression of the *cre* recombinase gene in postnatal forebrain neurons, and thus to cause selective depletion of striatal neuron A_{2A} receptors following brain development. We published (Bastia et al, 2005^[6]) evidence of successful forebrain-specific recombination by genetic, autoradiographic and behavioral assessments. We reported that during project Yr 2 that we found no consistent phenotype of the forebrain neuron A_{2A} receptor conditional KO (cKO) with respect to acute MPTP toxicity (see below).

In contrast, during Yr 3 we have found substantial attenuation of dopaminergic neuron injury in the forebrain neuron A_{2A} cKO mice in a more subacute toxin exposure (multiple smaller doses of MPTP administered over days) – potentially of greater pathophysiological relevance than the the acute/single high dose MPTP paradigm. In experiments conducted in collaboration with the laboratory of Prof. Micaela Morelli of the Univ. of Cagliari we found (**Fig. 1**) that the loss of dopaminergic (TH-IR) nigral neurons and the accompanying astrogliosis (GFAP-IR cell increase) was completely absent in littermate forebrain neuron A_{2A} cKO mice (i.e., mice homozygous for the *floxed* A_{2A} gene and also transgenic for the *cre* gene).



Exp# 3 – Brain A_{2A}R-dependence of caffeine's neuroprotective effect.

To explore this possibility in a chronic dual pesticide (paraquat + maneb) model of PD we first had to determine whether caffeine is protective in this model. C57Bl/6 mice were treated twice weekly with paraquat (Pq) and maneb (Mb) i.p. for 8 weeks. Ten min prior to each Pq+Mb toxin administration mice were pretreated with caffeine at 0 (saline vehicle), 5 or 20 mg/kg. Caffeine at the moderate dose of 20 mg/kg significantly attenuated the pesticide-induced loss of dopaminergic (TH+) nigral neurons, assessed by rigorous stereological methods. (See **Fig. 2A** and **Appendix A.**) By contrast, caffeine had no effect on TH- (non-TH immunoreactive) nigral cells (**Fig. 2B**), confirming the neuroprotective effect of caffeine (i.e., by excluding an induction of TH-immunoreactivity in originally TH- cells masking a loss of originally TH+ neurons in the mice pretreated with 20 mg/kg caffeine).



In Yr 3 we also initiated a study of the neuroprotective potential of urate, the end product of adenosine metabolism in humans, the immediate oxidation of xanthine, and a structural analog of caffeine (a.k.a. tri-methyl-xanthine). Urate is also a major antioxidant and is the main circulating antioxidant in humans in whom it circulates near the limits of solubility (and hence its pathophysiological role in urate crystal diseases like gout). Based on its antioxidant and chelating properties we and others have found urate to be the first molecular predictor of both risk of PD and the rate at which it progresses. (See **Appendix E** for further background.) We have provided the first evidence that urate can be neuroprotective in an *in vivo* model of PD (see **Appendix B**) by showing that locally administered (intracerebroventricular) urate can attenuate the toxicity of systemic MPTP.

Specific Aim #2 – to localize the region within brain through which caffeine or A_{2A} receptor inactivation produces its neuroprotective effect in the MPTP model of PD.

[Please see abstract publication in Appendix C.]

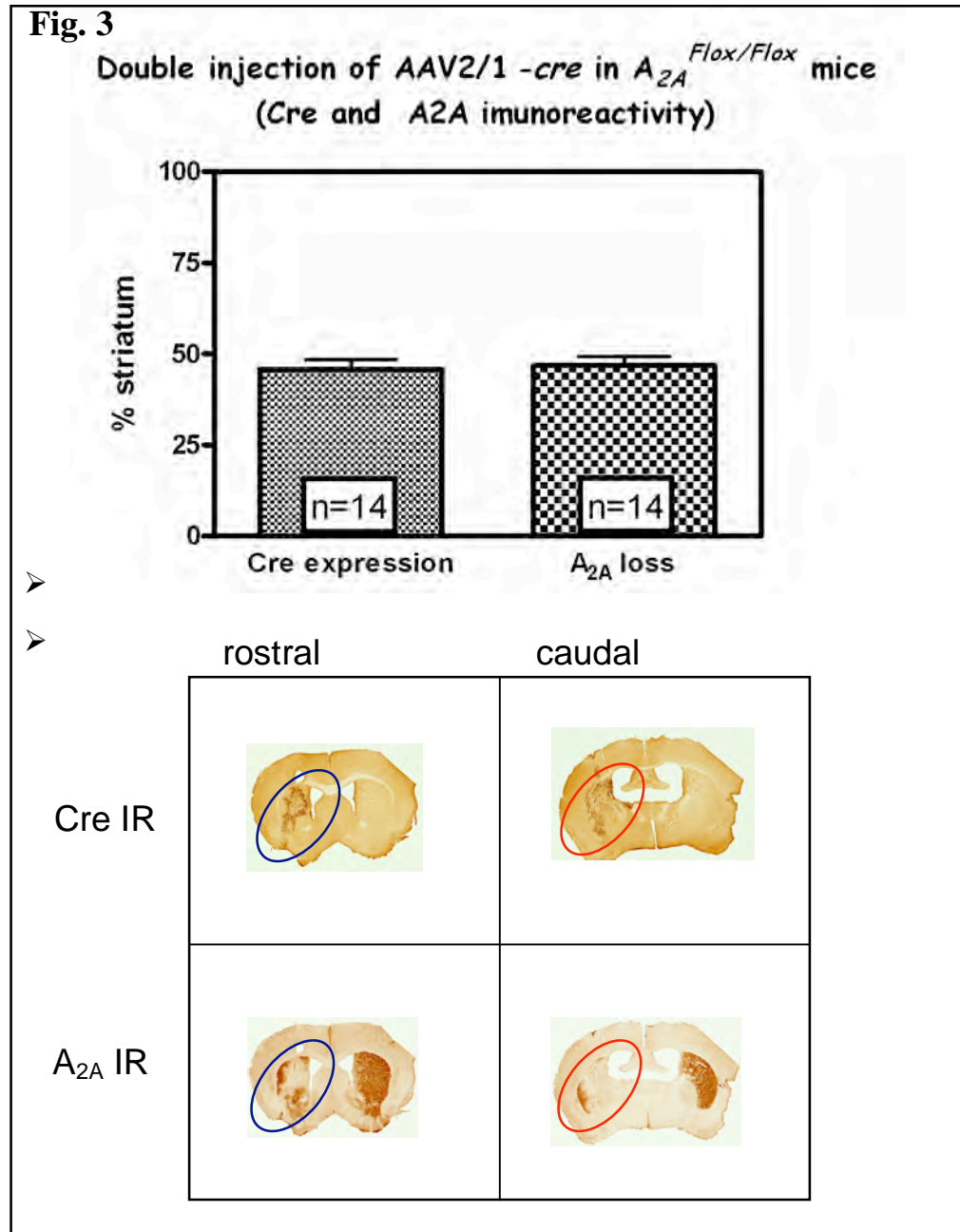
Hypothesis 2: Caffeine perfusion and focal A_{2A} receptors inactivation within striatum (but not frontal cortex) are sufficient to attenuate MPTP toxicity, by reducing toxin-induced striatal release of glutamate and/or GABA.

Exp# 5 – Effect of intracerebral infusion of Cre-expressing adeno-associated virus (AAV-Cre) on MPTP-induced toxicity in floxed A_{2A}R mice: Homozygous floxed A_{2A}R mice that previously received a stereotactic infusion of AAV-Cre or AAV-green fluorescent protein (AAV-GFP) into the striatum (or frontal cortex or substantia nigra) will be acutely exposed to systemic MPTP. One week later infusion needle track will be localized histochemically, while dopaminergic neuron integrity will be visualized by striatal DAT binding and nigral TH-IR counts will be assessed as in Exp #4.

As reported in Yr 2 we have characterized the time course and the dose-(titer-) dependence of unilateral local recombination and subsequent disruption of the A_{2A} gene in the viral Cre/*loxP* conditional KO method we adopted.

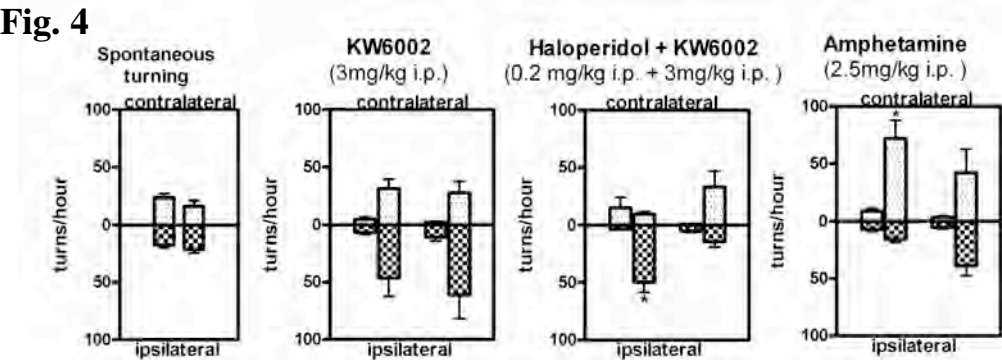
During Yr 3 we have further characterized the phenotype of this A_{2A}R cKO in the striatum of adult mice by testing the effect of unilateral striatal A_{2A}R loss in turning behavior and MPTP-induced loss of striatonigral dopaminergic neurons. Working with AAV2/1-*cre* and AAV2/1-*GFP* viruses (provided through a collaboration with Dr. Miguel Sena-Esteves of our institution), Dr. Augusta Pisanu has now demonstrated that:

- Using this AAV-Cre/*loxP* system, a double injection of AAV2/1-*cre* (3×10^{13} gc/ml) into the rostral and caudal striatum induces 50% of loss of A_{2A} striatal receptors in A_{2A}^{*fllox/fllox*} mice (**Fig 3**). This focal A_{2A}R KO phenotype was reached 30 days post injection and was still stable until 60 days post injection, although at that time cell toxicity was detected in the center of the infected area in some mice, probably related to an over-expression of Cre across time. (To reduce this late cell toxicity, in collaboration with Dr. Esteves, we are now testing a new and promising AAV vector, which delivers the Cre recombinase gene under control of a weaker promoter with the goal of eliminating toxicity.)



- This degree of unilateral loss of $A_{2A}R$ in mouse striatum is sufficient to induce a behavioral phenotype. Starting from the 30th day, to assess for asymmetries in striatal control of movement, turning behaviour was monitored in unilateral cKO mice ($AAV2/1\text{-cre}$, $A_{2A}R^{flx/flx}$) and in control mice ($AAV2/1\text{-GFP}$, $A_{2A}R^{flx/flx}$). None of the experimental groups showed a biased spontaneous turning behaviour. In unilateral conditional KO mice the A_{2A} antagonist KW-6002 (3 mg/kg, ip; in the presence of a sub-threshold dose of the dopamine D_2 antagonist haloperidol) induced ipsilateral turning, and amphetamine (2.5 mg/kg i.p.

on its own) induced contralateral turning relative to that in the control animals (Fig. 4).



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- AAV2/1-*cre* injection into the striatum of $A_{2A}R^{flox/flox}$ mice conferred a partial but significant neuroprotection against acute MPTP intoxication. 50 days after infection AAV2/1-*cre* injected mice and their respective AAV2/1-*GFP* controls were treated with MPTP or saline (4 times 15mg/kg i.p every 2 hours). Seven days after, the TH immunoreactivity of sparing projections in the striatum was measured: the AAV2/1-*cre*, $A_{2A}R^{flox/flox}$ mice showed a more intense TH staining in the infected side, compared to the intact one, while in the AAV2/1-*GFP*, $A_{2A}R^{flox/flox}$ group no difference was detectable (Figs. 5 and 6). Further stereological analysis of sparing neurons in *substantia nigra pars compacta* is now in progress, to confirm that focal disruption of A_{2A} receptor in the striatum is able to confer neuroprotection to dopaminergic neurons in the MPTP model of PD.

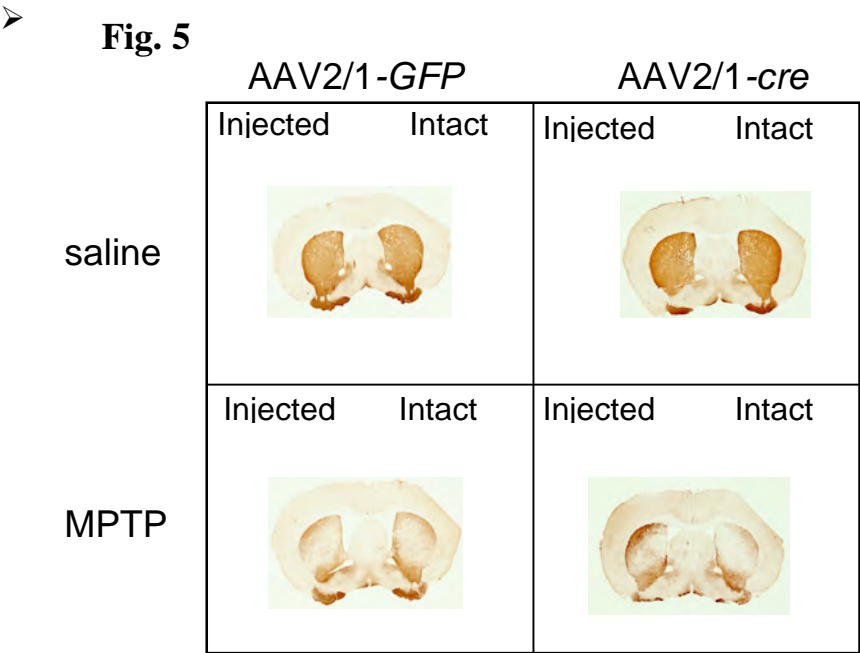
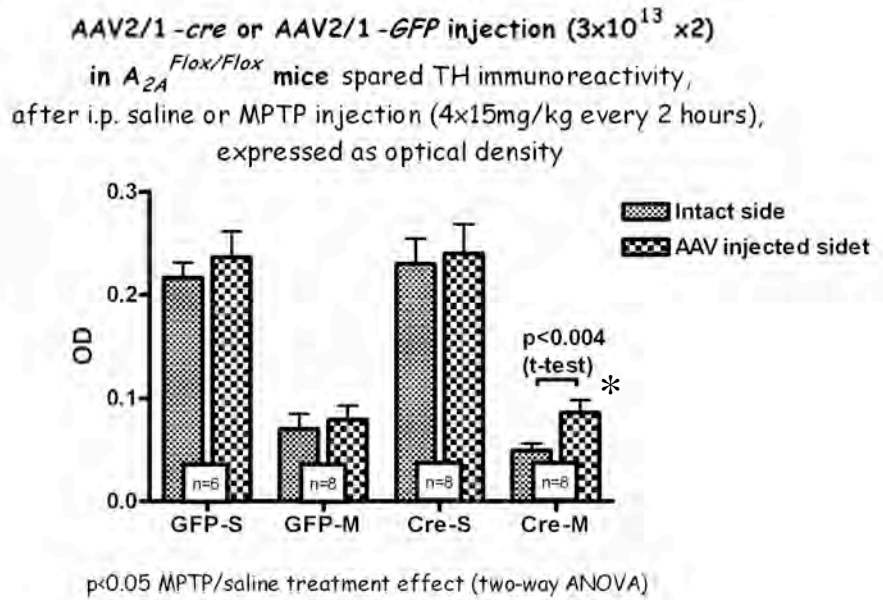


Fig. 6

- This methodological advance will allow us to dissect A_{2A} receptor involvement in neurotoxin models of PD with an unprecedented combination of anatomical and molecular precision in different brain structures.

Methods (for Figs. 3-6)

Mice with a *floxed* A_{2A} R gene were generated by insertion of *loxP* sequences within the introns flanking the exon 2 of the A_{2A} R gene (YJ Day and J linden, unpublished results). Homozygous adult male mice were anesthetized with an i.p. injection of Avertin and positioned in a stereotaxic frame for double injection into the left striatum (rostral: AP: +1.2; ML: +1.5; DV: -2.75; caudal: AP: -0.15; ML: +2.5; DV: -2.65) of $1 \mu\text{l}$ of AAV2/1-cre or AAV2/1-GFP (3×10^{13} gc/ml) at a rate of $0.1 \mu\text{l}/\text{min}$ by using a 30 gauge needle connected to a $50 \mu\text{l}$ Hamilton syringe driven by a microinfusion pump.

After 30 days mice were tested for asymmetries in striatal control of movement by recording contralateral and ipsilateral turns during the dark phase of their circadian rhythm, using an automated rotometry system (San Diego Instruments, San Diego, CA). Each mouse was placed at the center of 1 of 12 opaque glass flat-bottom bowls (10 cm diameter base, 13 cm high wall, with opening diameter of 26 cm) and connected to the lower end of a customized cable tether by a rubber band snugly fitted around the chest. The upper end of the cable is attached to a swivel box, which in turn is linked to a computer interface.

The 50th day the animals were randomly divided in 4 groups and received MPTP or saline injections (4 time 15mg/kg i.p. MPTP-HCL or saline every 2 hour) and 7 days later they were anesthetized and intracardially perfused with 10

ml of ice-cold saline followed by 30 ml of 4% paraphormaldehyde in 0.1 M phosphate buffer. After perfusion brains were removed and incubated overnight in the same fixative than cryoprotected by incubation in phosphate buffered 30% sucrose. Serial 25 μ m-thick coronal sections were cut on a freezing microtome and collected in 50 mM Tris buffer. Adjacent sections starting from the rostral part of the Striatum to the Substantia Nigra pars compacta (Snc) were collected for immunohistochemical staining for Cre recombinase (1:2000 Novagen anti-Cre rabbit polyclonal antibody) A_{2A}R (1:200 Santa Cruz anti A_{2A}R goat polyclonal antibody) and Tyrosine Hydroxylase (1:1000 Biomol anti-TH rabbit polyclonal antibody). Goat or Horse anti-species antibodies conjugated to biotin, Vectastain ABC Kit and fast DAB Kit were used for detecting primary antibodies. Immunostaining controls were done without the primary antibody. Quantification of Cre and A_{2A}R-immunoreactivity (IR) was performed every sixth section at 4X magnification by measuring the extent of the immunopositive or immunonegative area in the Striatum. TH-immunoreactivity was quantified every 12 sections at 4X magnification by measuring the Optical Density (OD) of the striatal area, corrected by the OD of overlying cortex. as background. Statistical analysis was performed by Oneway ANOVA followed by a Tukey post hoc analysis of means difference between groups. Student's t test was used for the remaining statistical analyses. A value of p < 0.05 was considered to be significant.

Specific Aim #3 – to investigate caffeine-estrogen interactions in the MPTP model of PD by determining the effect estrogen replacement on the neuroprotective phenotype of A_{2A} KO mice, and exploring potential peripheral and CNS mechanisms contributing to caffeine's reduced neuroprotective efficacy in the presence of estrogen.

[Please see publication in Appendix D, a translational neuroscience review focused on developing neuroprotective therapeutics for PD, with caffeine and estrogen included in the context of broader strategies.]

With support from this award we have now fully completed and published our studies of estrogen-caffeine interaction in the MPTP model of Parkinson's disease, as detailed in our Yr 2 progress reported. Our results demonstrate that estrogen reduces caffeine's neuroprotective effect against MPTP toxicity in both male and female mice. In the context of human epidemiology on PD, our findings suggest a biological basis for the interaction between estrogen and caffeine in modifying the risk of PD.

Key Research Accomplishments (in Year 3)

- Demonstration that neuronal forebrain A_{2A} receptors can play a critical role in dopaminergic neuron injury in the MPTP model of neurodegeneration in Parkinson's disease.
- Using a powerful newly Cre/LoxP conditional knockout system, we have obtained evidence that it is the neuronal forebrain A_{2A} receptors in the striatum that are responsible for this toxicity. Thus it is through these receptors that caffeine and more specific antagonists of the adenosine A_{2A} receptor may offer neuroprotection against the development or progression of PD.
- Demonstration for the first time that caffeine's neuroprotective effect extends to the dual pesticide – paraquat plus maneb – model of PD, a chronic, potentially more environmentally relevant model of the disease.
- Demonstration for the first time that urate can be neuroprotective in an *in vivo* model of PD. This finding may have a particularly rapid translational impact as urate-elevating therapy is now being pursued as potential neuroprotectant for PD patients.
- Methodological advances were achieved with a viral vector-based Cre/LoxP conditional knockout system. It will allow us to dissect caffeine and A_{2A} receptor involvement in neurotoxin models of PD with an unprecedented combination of anatomical and molecular precision in different brain structures.

Reportable Outcomes

1) Publications (with acknowledgements citing W81XWH-04-1-0881/ USAMRAA)

- Kachroo A, Prasad K, Irizarry MC, Richfield EK, Schwarzschild MA. 2007. Caffeine protects against combined paraquat and maneb-induced neurotoxicity of dopaminergic nigral neurons. 2007 Neuroscience Meeting. San Diego, CA: Society for Neuroscience. Abstract # 265.20/U10. [See **Appendix A.**]
- Luo W, Schwarzschild MA, Xu K. 2007. Urate attenuates MPTP-induced dopaminergic neurotoxicity in mice. 2007 Neuroscience Meeting. San Diego, CA: Society for Neuroscience. Abstract # 797.8/O12. [See **Appendix B.**]
- Pisanu A, Sena-Esteves M, Schwarzschild MA. 2007. Behavioural phenotype of unilateral conditional knockout of striatal adenosine A_{2A} receptors using a viral Cre/loxP system. 2007 Neuroscience Meeting. San Diego, CA: Society for Neuroscience. Abstract # 314.7/GGG27. [See **Appendix C.**]
- Hung AY, Schwarzschild MA. 2007. Clinical trials for neuroprotection in Parkinson's disease: overcoming angst and futility? *Curr Opin Neurol.* 20:477-483. [See **Appendix D.**]

2) Major presentations (with support from/acknowledgements including DoD/USAMRAA/NETRP)

- October 18, 2006 – Society for Neuroscience annual meeting (Atlanta)
Symposium: *Purinergic Signaling in Neuron-Glia Interactions* “Adenosine A_{2A} Receptors in Neurodegeneration” {Eweson Lectureship}.
- April 5, 2007, 5th Annual NIEHS/CCPDER Meeting (Asilomar/Monterey)
“Purines & Parkinson’s: Pursuing environmental exposures linked to reduced risk” {keynote speaker}.
- June 27, 2007, Parkinson’s Disease and Environment Consensus Conference (Sunnyvale, CA) “Metabolic Concerns: Serum urate predicts progression of Parkinson’s disease” {panel presenter}.
- July 15, 2007 – International Brain Research Organization (IBRO) World Congress of Neuroscience. (Melbourne) Symposium: “*Role of adenosine A_{2A} receptors in noxious brain conditions: effects on neurons, astrocytes or microglia?*” {chair; presenter}.

Conclusions

Central hypothesis: Multiple environmental protectants and toxins interact to influence of the health of the dopaminergic neurons lost in Parkinson’s disease. Our progress under this award supports the central hypothesis, particularly with respect to caffeine-estrogen interactions in models of PD (**SA 3**). Critical to our ability to successfully pursue the Specific Aims of our research program, we have made substantial progress in establishing and characterizing the key KO methodologies of this project, and now have initial data applying this method to the MPTP model of neurodegeneration in PD. We expect to build on our conceptual and technical advances of the first three years in our final, publishing many of the preliminary data reported here.

References

- ^[1]Institute of Medicine. (2003) Gulf War and Health: Volume 2. Insecticides and Solvents. (Washington, DC: National Academies Press), 1-632.
- ^[2]Charatan F. (2002) US links motor neurone disease with Gulf war service. *BMJ*. 2002 324:65.
- ^[3]Haley RW et al. Effect of basal ganglia injury on central dopamine activity in Gulf War syndrome: correlation of proton magnetic resonance spectroscopy and plasma homovanillic acid levels. *Arch Neurol*. (2000) 57:1280-1285.
- ^[4]Karen DJ et al. (2001) Striatal dopaminergic pathways as a target for the insecticides permethrin and chlorpyrifos. *Neurotoxicology* 22:811-817.
- ^[5]Lamberg, L. (1999) Brew It or Chew It? Military Seeks Ways to Caffeinate *JAMA* 281:885-886.
- ^[6]Bastia, E., Xu, Y. -H., Scibelli, A. C., Day, Y. -J., Linden, J., Chen, J. -F., & Schwarzschild, M. A. (2005). A crucial role for forebrain adenosine A2A receptors in amphetamine sensitization. *Neuropsychopharmacology*, 30:891-900.
- ^[7]Li, X. X., Nomura, T., Aihara, H., & Nishizaki, T. (2001). Adenosine enhances glial glutamate efflux via A2A adenosine receptors. *Life Sci* 68, 1343– 1350.
- ^[8]Nishizaki, T., Nagai, K., Nomura, T., Tada, H., Kanno, T., Tozaki, H., et al. (2002). A new neuromodulatory pathway with a glial contribution mediated via A(2a) adenosine receptors. *Glia* 39, 133– 147.

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Program#/Poster#: 265.20/U10
Title: Caffeine protects against combined paraquat and maneb-induced neurotoxicity of dopaminergic nigral neurons
Location: San Diego Convention Center: Halls B-H
Presentation Start/End Time: Sunday, Nov 04, 2007, 3:00 PM - 4:00 PM
Authors: ***A. KACHROO**¹, K. PRASAD², M. C. IRIZARRY¹, E. K. RICHFIELD², M. A. SCHWARZSCHILD¹;
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The etiology of idiopathic Parkinson's disease (PD) remains unknown though certain risk factors have been identified. On the one hand, genetic determinants and environmental neurotoxicants such as pesticides have been linked to an increased risk of developing PD. On the other hand, several environmental factors such as caffeine intake have been inversely linked to PD risk. The present study sought to determine whether caffeine (a non-specific adenosine antagonist) attenuates dopamine neuron toxicity from combined exposure to the herbicide paraquat (PQ) and the fungicide maneb (MB). The intraperitoneal injection paradigm involved dual administration of 10mg/kg PQ and 30mg/kg MB, in the absence or presence of caffeine at 5mg/kg and 20mg/kg (injected 10 min prior to pesticides), twice a week for 8 weeks. PQ/MB treated mice did not demonstrate any motor deficits when tested 24-48 h post injection, using open field locomotor activity. Stereological assessment of neurons in the substantia nigra pars compacta was performed using tissue sections stained for tyrosine hydroxylase (TH) and counterstained with cresyl violet. The data showed that the numbers of TH-positive (dopaminergic) nigral neurons were significantly reduced (40%) after paraquat and maneb treatment. Pretreatment with caffeine at 20mg/kg, but not 5mg/kg, provided significant protection against TH-positive neuronal cell loss. The lack of a significant difference observed between control and pesticide-treated groups when counting TH-negative (non-dopaminergic) neurons confirms the specificity of these pesticides for targeting dopaminergic neurons. These data demonstrate that caffeine is able to neuroprotect dopaminergic nigral neurons in the setting of an environmentally relevant model of PD.

Disclosures: **A. Kachroo**, None; **K. Prasad**, None; **M.C. Irizarry**, None; **E.K. Richfield**, None; **M.A. Schwarzschild**, None.

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Program#/Poster#: 797.8/O12
Title: Urate attenuates MPTP-induced dopaminergic neurotoxicity in mice
Location: San Diego Convention Center: Halls B-H
Presentation Start/End Time: Wednesday, Nov 07, 2007, 11:00 AM -12:00 PM
Authors: **W. LUO**^{1,2}, M. A. SCHWARZSCHILD², *K. XU²;
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Findings from large prospectively followed populations have firmly linked higher urate level with a reduced risk of developing Parkinson's disease (PD). Recently, higher serum urate has been identified as a predictor of slower progression of PD symptoms and signs in two long-term, rigorously conducted clinical trials (together comprising over 1600 early cases of PD). These observations raise the possibility of a novel neuroprotective effect of urate in PD, and prompted us to explore its role in an animal model of PD. We examined the effect of locally administered urate on MPTP toxicity in young male C57Bl/6 mice. Urate (0, 2.5, 5, 7.5, or 10 mg/dL in artificial CSF) was perfused through microdialysis probe within a guide canula into the left lateral ventricle of free moving mice for 48 hours. MPTP-HCl (single ip injection of 40 mg/kg) or saline was given 24 hours after the start of urate perfusion. One week after MPTP, striatal dopamine content was determined. Urate (5mg/dL but not at higher dose) perfusion significantly attenuated MPTP-induced striatal dopamine loss. These preliminary data support a neuroprotective role of urate in the MPTP mouse model of PD.

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Program#/Poster#: 314.7/GGG27
Title: Behavioural phenotype of unilateral conditional knockout of striatal adenosine A_{2A} receptors using a viral Cre/loxP system
Location: San Diego Convention Center: Halls B-H
Presentation Start/End Time: Sunday, Nov 04, 2007, 3:00 PM - 4:00 PM
Authors: *A. PISANU, M. SENA-ESTEVEES, M. A. SCHWARZSCHILD;
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Genetic knockout (KO) approaches to the study of receptor function complement traditional pharmacological methods by offering complete specificity and inactivation. However, standard KO strategies globally eliminate the targeted receptor starting prenatally, and thus their use for investigating the role of receptors in the adult brain can be confounded by developmental or systemic phenotypes. The transgenic Cre/loxP conditional KO system can achieve partial control over the timing and distribution of receptor inactivation using a specific promoter to direct Cre recombinase gene (*cre*) expression postnatally and/or in a selected cell subtype. A_{2A} receptors (A_{2A}R) have been reported to modulate motor functions in basal ganglia circuitry and their antagonists possess anti-parkinsonian activity in animal models. Convergent epidemiological and laboratory data have also suggested that A_{2A}R blockade may confer neuroprotection against the dopaminergic neuron degeneration that causes Parkinson's Disease. Stereotaxic infusion of an adeno-associated virus (AAV) vector encoding Cre recombinase into the left striatum of homozygous floxed A_{2A}R (A_{2A}^{R^{flox/flox}}) adult mice was used to analyze the role of striatal A_{2A}R in motor control and neuroprotection. Neuronal Cre expression and A_{2A}R loss were visualized by immunohistochemistry and correlated with turning behaviour to assess for asymmetries in striatal control of movement. Cre expression appeared maximal 15 days post-infusion of AAV2/1-*cre* vector whereas loss of A_{2A}R immunoreactivity reached its maximum extent at 30 days post-injection. Starting from the 30th day turning behaviour was assessed in unilateral conditional KO mice (AAV2/1-*cre*, A_{2A}^{R^{flox/flox}}) and in two control groups (AAV2/1-*GFP*, A_{2A}^{R^{flox/flox}} and AAV2/1-*cre*, A_{2A}^{R^{+/+}} mice). None of the experimental groups showed a biased spontaneous turning behaviour. In unilateral conditional KO mice the A_{2A} antagonist KW-6002 (3 mg/kg, ip; in the presence of a sub-threshold dose of the dopamine D₂ antagonist haloperidol) induced ipsilateral turning, and amphetamine (2.5 mg/kg i.p. on its own) induced contralateral turning relative to that in the control animals. Thus the AAV2/1-Cre/loxP conditional KO system provides a means to precisely eliminate the A_{2A}R postnatally in discrete brain regions, and can be used to further explore the neurobiology of adenosine receptors and their pathophysiology in models of CNS disease.

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Clinical trials for neuroprotection in Parkinson's disease: overcoming angst and futility?

Albert Y. Hung^a and Michael A. Schwarzschild^{a,b}

Purpose of review

To summarize recently published results of neuroprotection trials for Parkinson's disease, and discuss them in the context of evolving concepts in clinical study design and animal models.

Recent findings

Despite compelling preclinical evidence from laboratory models suggesting potential neuroprotective benefits, the antioxidant, antiapoptotic, antiexcitotoxic, immunomodulatory and neurotrophic agents studied to date have not shown clear benefit in human studies. The futility study design, an alternative approach focused on efficiently excluding less promising compounds, has been adopted recently to investigate four candidate neuroprotectants. A delayed-start trial design has also been introduced in a study of the monoamine oxidase inhibitor rasagiline, demonstrating a possible neuroprotective effect as well as its clear symptomatic benefit. In parallel with these clinical innovations, preclinical research initiatives are identifying new animal models that more closely resemble the clinical course and pathology of Parkinson's disease.

Summary

Angst over disappointing results of neuroprotection trials in Parkinson's disease has engendered efforts to refine animal models at one end of the therapeutics pipeline, and to optimize clinical trial design at the other. Building on new insights into the genetics, epidemiology and pathogenesis of Parkinson's disease, these recent improvements in 'translational infrastructure' will enhance the prospects of achieving the critical goal of slowing the progression of disability.

Keywords

clinical trial, futility study, neuroprotection, Parkinson's disease

Abbreviations

6-OHDA	6-hydroxydopamine
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
SPECT	single photon emission computed tomography
UPDRS	Unified Parkinson's Disease Rating Scale

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Introduction

Parkinson's disease is characterized clinically by the cardinal features of resting tremor, rigidity, bradykinesia, and gait difficulty with postural instability. Motor dysfunction results from the progressive and selective loss of dopaminergic neurons in the substantia nigra pars compacta. Highly effective symptomatic treatments, primarily based on dopamine replacement strategies, are available to treat early to moderate stage Parkinson's disease, providing significant improvement in motor function and quality of life. Despite advances in understanding of the pathogenesis of Parkinson's disease, however, efforts to identify a therapy to slow or reverse disease progression have been disappointing. Indeed, a recent practice parameter submitted by the American Academy of Neurology concluded that 'no treatment has been shown to be neuroprotective' [1^{••}]. Thus, clinical and basic research focused on identifying these neuroprotective strategies addresses a major unmet need [2[•]].

The etiology of idiopathic Parkinson's disease remains uncertain. A number of genetic mutations have recently been identified in some patients with a familial form of the disorder (reviewed elsewhere in this volume). These mutations, however, account for only a small percentage of cases of Parkinson's disease. In addition, twin studies suggest that while genetic factors may predispose young-onset patients to Parkinson's disease, they appear to be less important in the majority of individuals with later onset disease [3]. Indeed, current models propose a complex interplay of biochemical and cellular processes, including oxidative stress, mitochondrial dysfunction, excitotoxicity, inflammation, trophic factor deficiency, and apoptosis [4,5]. In light of the significant clinical impact that would be achieved by identifying agents slowing disease progression, the Committee to Identify Neuroprotective Agents in Parkinson's (CINAPS) was formed and assigned the task of prioritizing prospective neuroprotective candidates for clinical trials [6]. Four primary criteria were used: scientific rationale, evidence

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Table 1 Putative neuroprotective agents identified by the Committee to Identify Neuroprotective Agents in Parkinson's (CINAPS) group

Agent	Primary mechanism
Caffeine	Adenosine antagonist
Coenzyme Q10	Antioxidant/mitochondrial stabilizer
Creatine	Antioxidant/mitochondrial stabilizer
Estrogen	Unknown
GM-1 ganglioside	Trophic factor
Minocycline	Antiinflammatory/antiapoptotic
Nicotine	Unknown
GPI-1485	Trophic factor
Pramipexole	Antioxidant
Rasagiline	Antioxidant/antiapoptotic
Ropinirole	Antioxidant
Selegiline	Antioxidant/antiapoptotic

Data from [6].

of blood–brain barrier penetration, adequate safety and tolerability data, and efficacy in animal models and preliminary human studies. Based on these criteria, 12 compounds were selected as attractive candidates for further study (Table 1). Here, we review the recent clinical trials focused on these and other agents, and discuss the limitations of current clinical study design and preclinical animal models.

Lessons from early neuroprotective trials

Clinical neuroprotection trials for Parkinson's disease date back to the early 1980s, when oxidative stress and oxidative activation of putative environmental protoxins were first proposed as mechanisms for dopamine neuron loss. On the basis of this hypothesis, early trials focused on the potential effectiveness of selegiline, a selective, irreversible inhibitor of monoamine oxidase type B (MAO-B) that had been shown to protect against dopamine cell loss in animal models (Table 2) [7–16,17•, 18•,19•,20–26,27•,28], reviewed in [29•]. Using defined clinical endpoints [time until symptomatic treatment was required, or changes in the Unified Parkinson's Disease Rating Scale (UPDRS)], these studies demonstrated a clear benefit in those who received selegiline. It became evident, however, that these results could similarly be explained by a symptomatic effect of the study intervention.

In order to overcome the confounding effects of symptomatic benefit, several studies employed radiotracer imaging as a surrogate measure of dopaminergic nigrostriatal neuron integrity. ^{123}I - β -CIT single photon emission computed tomography (SPECT) reflects binding to the dopamine transporter on dopaminergic cells, and [^{18}F]fluorodopa PET measures the uptake and conversion of the tracer into fluorodopamine in surviving neurons. This approach was used to study the potential neuroprotective effects of the nonergot dopamine agonists pramipexole and ropinirole [20,21]. Both of these agents (compared with levodopa) resulted in less

reduction in uptake of the putative biomarker over the course of the treatment period, suggesting a possible neuroprotective effect with agonist therapy. The ELLDOPA study [22], investigating the effects of levodopa on disease progression, has brought the reliability of imaging measures into question. In this study, patients treated with carbidopa-levodopa showed a smaller decrease in UPDRS scores, but a significantly greater decline in β -CIT uptake. Thus, as radiotracer imaging does not consistently correlate with clinical endpoints, it has not been established as an appropriate surrogate of neurodegeneration in neuroprotection trials for Parkinson's disease [30].

Antioxidants

The focus on oxidative stress as a mechanism of dopaminergic cell loss has led to several additional trials examining whether antioxidant therapy can modify the course of Parkinson's disease. Coenzyme Q₁₀ is a cofactor in the electron transport chain, and has potent antioxidant effects. It is also thought to stabilize mitochondrial function. In a pilot study of coenzyme Q₁₀ in early Parkinson's disease, a total of 80 patients were assigned to placebo or one of three different doses (300, 600 and 1200 mg per day) [16]. Using change in UPDRS as the primary outcome measure, there appeared to be an inverse trend between dose and degree of clinical change, suggesting possible neuroprotection. The potential benefit of coenzyme Q₁₀ was most clearly suggested at the highest dose, though at this and other doses there was no significant effect on secondary measures of a timed tapping test of motor function or of the time until levodopa therapy initiation was warranted. Since that study, daily doses as high as 2400 mg have been shown to be well tolerated [31].

Creatine is a dietary supplement that plays an important role in mitochondrial ATP production. In animal models of Parkinson's disease, oral creatine supplementation has been shown to have a neuroprotective effect [32]. The results from a 2-year placebo-controlled pilot efficacy trial were recently reported [18•]. Changes in dopamine transporter SPECT were used as the primary outcome measure, and the UPDRS and 36-item Short Form Health Survey (SF-36) were secondary outcome measures for disease progression. In this study, creatine supplementation had no significant effect on SPECT variables, nor did it result in a change in overall UPDRS scores. With creatine, there was a slight improvement in mood (as assessed by UPDRS subscale I, 'Mentation, behavior, mood'). There was also a lower relative increase in agonist dose in patients treated with creatine, although this reflected primarily a difference in baseline dose rather than final dose. The authors concluded, however, that the negative results could potentially be related to the low doses used in the study.

Table 2 Published randomized neuroprotection trials in Parkinson's disease

Class	Agent	n	Primary outcome	Duration	Comments	Reference
Antioxidant/MAO inhibitor	Selegiline	800	Time to symptomatic treatment		DATATOP	[7]
	Selegiline	54	Time to symptomatic treatment	3 years		[8]
	Selegiline	157	Time to symptomatic treatment	1–3 years		[9]
	Selegiline	101	Change in UPDRS	14 months	2 month washout	[10]
	Selegiline	79	Change in UPDRS	60 months	1 month washout	[11]
	Lazabemide	321	Time to symptomatic treatment	12 months		[12]
	Rasagiline	404	Change in UPDRS	12 months	Delayed start	[13]
	Selegiline	157	Change in UPDRS	7 years	With levodopa	[14]
Antioxidant	Vitamin E	800	Time to symptomatic treatment		DATATOP	[15]
	Coenzyme Q10	80	Change in UPDRS	16 months	OE2	[16]
	Coenzyme Q10	213	Change in UPDRS	12 months	Futility study	[17**]
	Creatine	60	[¹²³ I]-FP-CIT SPECT	24 months		[18*]
	Creatine	200	Change in UPDRS	12 months	Futility study	[19*]
DA replacement	Pramipexole	82	[¹²³ I]-β-CIT SPECT	46 months	CALM-PD	[20]
	Ropinirole	186	[¹⁸ F]-dopa PET	24 months	REAL-PET	[21]
	Levodopa	360	Change in UPDRS	40 weeks	ELLDOPA;	[22]
					2 week washout	
Glutamate antagonist	Riluzole	20	Change in UPDRS	6 months		[23]
Trophic factor	GDNF	50	Change in UPDRS motor score	8 months		[24]
	GDNF	34	Change in UPDRS motor score	6 months		[25]
Neuroimmunophilin	GPI-1485	300	Change in UPDRS motor score	6 months		Discussed in [26]
ligand	GPI-1485	213	Time to symptomatic treatment	12 months	Futility study	[17**]
Antiapoptotic agent	TCH346	301	Change in UPDRS	12–18 months		[27**]
	CEP-1347	806	Change in UPDRS	Average 21.4 months	PRECEPT	[28]
	Minocycline	200	Time to symptomatic treatment	12 months	Futility study	[19*]

MAO, monoamine oxidase; UPDRS, Unified Parkinson's Disease Rating Scale; SPECT, single photon emission computed tomography; DA, dopamine; GDNF, glial cell line-derived neurotrophic factor.

As a step to assessing whether coenzyme Q₁₀ or creatine are suitable for further study as neuroprotective agents, phase II studies were conducted, concluding that neither agent could be rejected as futile (see below; [17**,19*]). On the basis of these data, larger multicenter study using coenzyme Q₁₀ and creatine are being planned or in progress.

Antiapoptotic agents

Apoptosis, or programmed cell death, has been proposed to contribute to the pathogenesis of neurodegenerative disorders, including Parkinson's disease, although the neuropathologic evidence remains somewhat controversial [33]. Despite this uncertainty, there are numerous reports to suggest that interfering with apoptotic pathways can protect against neuronal cell death [34–36]. One class of antiapoptotic agents, the propargylamines, has been proposed as neuroprotective agents [37*]. TCH346 (*N*-methyl-*N*-propargyl-10-aminomethyl-dibenzo[b,f]-oxepin; also called CGP3466) is a propargylamine that resembles structurally selegiline, but lacks MAO inhibitor activity. Its antiapoptotic mechanism of action is thought to involve binding to glyceraldehyde-3-phosphate [38]. In animal models of Parkinson's disease, the agent has been shown to protect against neurodegeneration and associated behavioral deficits [39,40]. A recent clinical study examined the putative neuroprotective effects of TCH346 on disease progression [27**].

Patients were randomly assigned to placebo or one of three treatment doses. After a 12–18-month treatment period and 4-week washout, they failed to show any difference in the primary endpoint (time to disability requiring dopaminergic therapy) or secondary endpoints (change in UPDRS or the Parkinson's disease questionnaire (PDQ)-39, a measure of quality of life).

CEP-1347 inhibits the mixed lineage kinases (MLKs) that activate the c-Jun N-terminal kinase (JNK) signaling cascade, a pathway that mediates apoptotic cell death. MLK inhibition and disruption of this pathway have consistently been shown in preclinical models to enhance neuronal survival [41,42]. These promising data prompted the PRECEPT trial, the largest neuroprotection trial completed to date, in which patients not yet requiring dopaminergic therapy were randomized to placebo or one of the doses of CEP-1347 [28]. Using time to disability requiring dopaminergic therapy as the primary endpoint, and changes in UPDRS and β-CIT SPECT imaging as secondary endpoints, this study was concluded early (average of 21.4 months of follow up) after an interim analysis showed that a greater percentage of patients on the lowest and highest doses of experimental drug (10 and 50 mg twice a day) reached the primary endpoint, compared with the placebo group. Similarly, all active treatment groups showed a greater decline in striatal β-CIT. Thus, CEP-1347 was shown to be ineffective

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at modifying disease progression in Parkinson's disease. Based on these negative clinical trials, the relevance of apoptosis to designing neuroprotective agents requires reassessment [43^{••}]. Alternatively, it has been suggested that apoptosis may contribute as just one of several routes to neuronal degeneration, such that blockade of apoptosis may lead to a different (e.g. necrotic) cell death pathway that may produce at least as much neuronal injury [44,45].

The 'delayed start' design

Recently, the use of a 'delayed start' design has been proposed for neuroprotection trials [13,46^{••}]. As noted, symptomatic effects of study interventions can confound interpretation when clinical measures are used as surrogates of disease progression. While this is addressed in part by incorporation of a 'washout' phase, when clinical performance is compared between untreated baseline and final visits, it is difficult to exclude the possibility that the treatment may have long-lasting effects that exceed the duration of the washout. Ethical issues have also been raised about withholding effective treatment during the washout period.

In the delayed start design, some study participants begin treatment with the experimental agent immediately, while others begin after a delay. This design presumes that the symptomatic benefit of the medication is similar in both groups at the end of treatment. Thus, any change in outcome measure should reflect a disease-modifying effect. The delayed start design has been used to study the effects of rasagiline, a propargylamine MAO-B inhibitor [13] that has been shown to protect against 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic cell loss in animal models [47]. In an initial 6-month placebo-controlled phase, treatment with rasagiline resulted in improvement in Parkinsonian symptoms [48]. Following this phase, study participants previously treated with placebo were given rasagiline (2 mg per day) and compared with individuals taking rasagiline from the start. Those treated with rasagiline (1 or 2 mg daily) for 12 months showed a lesser decline in clinical performance (as measured by change in UPDRS scores) than those only taking the medication for 6 months. This study has been interpreted to suggest that rasagiline can slow the rate of disease progression, prompting a similar but longer and larger study now underway [49].

The use of futility studies

The failure of agents proven effective in preclinical studies to have any efficacy in human studies has led clinical investigators to ask whether there is a more efficient way to screen out compounds that are ineffective as neuroprotectants. In the last several years, a futility study design has been used to assess several prospective neuroprotective agents [50,51^{••},52[•]]. This methodology has been used previously to evaluate cancer treatments

and more recently, stroke treatments. Phase II pilot studies with such a design require a smaller number of patients over a shorter time period than large phase III efficacy studies. They focus on distinguishing between potentially effective agents appropriate for larger randomized studies, and agents that are demonstrably 'futile' (at least at the dose tested). In order for such an approach to be effective, it is necessary to identify clinical outcome measures that would be expected to change sufficiently in untreated patients over 6–12 months, the short time period intended in futility studies. An analysis of historical Parkinson's disease data sets identified change in total UPDRS or change in motor plus activities of daily living (ADL) UPDRS (subscales 2 and 3) as requiring the smallest sample sizes [50]. While some efficacy data can be obtained from such studies, however, they lack (by design) sufficient statistical power to test whether a drug is actually disease modifying.

A randomized, double-blind futility trial was conducted by the National Institutes of Health (NIH) Exploratory Trials in Parkinson's disease (NET-PD) program, examining a potential neuroprotective effect of creatine and minocycline [19[•]]. Minocycline is a semi-synthetic second-generation tetracycline that has been proposed to protect against MPTP-induced dopaminergic cell loss via its antiinflammatory action [53]. A total of 200 participants were randomized to receive either placebo, creatine (10 g per day), or minocycline (200 mg per day). Using change in total UPDRS as the primary endpoint, neither agent could be rejected as futile. All three treatment groups were fairly well tolerated (91% in creatine group, 77% in minocycline group); the most common adverse events included upper respiratory symptoms (26%), joint pain (19%), and nausea (17%).

Neuroimmunophilins are a family of proteins present in the nervous system that were initially identified as targets of the immunosuppressive agents FK506 and rapamycin [26,54]. The neuroimmunophilin ligand GPI-1046 has been shown to reverse the loss of corticostriatal long-term potentiation in 6-hydroxydopamine lesioned rodents, suggesting that it may protect dopaminergic cell function [55]. An initial study using a similar agent, GPI-1485, at a maximal dose of 4000 mg daily showed no treatment benefit on the primary outcome of change in UPDRS motor score [26]. To assess whether GPI-1485 deserves further study, the agent was tested along with coenzyme Q₁₀ using the futility trial design [17^{••}]. In this study, a total of 213 individuals were randomized to placebo, coenzyme Q₁₀ (2400 mg per day), or GPI-1485 (4000 mg per day). Over a 12-month trial period (or until symptomatic therapy was warranted), the primary outcome measure (change in total UPDRS scores) again did not meet the prespecified criteria for futility of either agent. Like the other two agents studied, the study

interventions were well tolerated, with a similar set of adverse effects.

Based on these studies, all four agents tested remain potential candidates for larger phase III trials. Several important issues, however, have been raised by these reports. The placebo group in the creatine/minocycline study showed a mean change in total UPDRS of 8.39, whereas the coenzyme Q10/GPI-1485 placebo group showed a much smaller change (6.31). When the four treatment arms were analyzed in comparison to the combined mean of both placebo groups and the placebo arm of the PRECEPT trial, only creatine was not found to be futile (i.e. potentially disease modifying). Thus, the study conclusion depends on the placebo data used to determine the futility threshold. This raises the concern that changes in UPDRS, the primary response variable used in these trials, may be an inadequate primary outcome measure. It may be necessary to employ multiple outcome measures or global statistical tests [46^{••}].

Limitation of preclinical rationales: animal models

The recent negative results from clinical trials of a small but diverse set of potential neuroprotectants may reflect a series of individual inadequacies in the identification of candidate drugs or in the design and execution of clinical trials for neuroprotection in Parkinson's disease. Alternatively, they could reflect a broader systematic problem in our identification or testing of candidate agents. Accordingly, in addition to clinical trial design, the adequacy of available animal models has also come into question. Short of being confirmed as predictive of a drug's neuroprotective potential in humans, an animal model of neurodegeneration can be validated by demonstrating that it shares the progressive nature of the disease and the defining pathologic features. Most preclinical studies have used the MPTP or 6-hydroxydopamine (6-OHDA) model to characterize protective effects on dopaminergic cell loss [56]. Indeed, results from these models served as one of the primary evaluation criteria for inclusion in the list of attractive candidates by CINAPS [6]. MPTP is a mitochondrial complex I inhibitor, damaging dopamine neurons in part through the generation of reactive oxygen species. 6-OHDA, when administered intracerebrally, also induces oxidative injury by entering cells through catecholamine transporters. Nevertheless, these models have limitations [57]. The 6-OHDA model causes acute degeneration of nigrostriatal neurons, and does not cause the pathognomonic cytoplasmic inclusions (Lewy bodies) seen in Parkinson's disease. Similarly, most MPTP protocols use acute treatments and fail to produce Lewy bodies, though Lewy body-like cytoplasmic inclusions have been reported in a chronic MPTP infusion model [58]. Moreover, the effects of putative neuroprotectants have been shown to vary

depending on the toxin administration protocol [59[•]]. In an effort to enhance the environmental relevance of toxin models of Parkinson's disease, the pesticide rotenone and herbicide paraquat have been used in toxin models of Parkinson's disease. While some reports indicate a chronic, progressive loss of nigrostriatal neurons, inconsistent pathologic effects have undermined their utility for testing neuroprotective compounds [60].

Recent genetic data have implicated dysfunction in the ubiquitin–proteasome system in the pathogenesis of Parkinson's disease (reviewed elsewhere in this volume). This led McNaught and colleagues [61,62[•]] to investigate the effect of systemic exposure to proteasome inhibitors. They reported that rodents treated with epoxomicin or PSI, two different proteasome inhibitors, for 2 weeks produced a progressive dopamine-responsive neurologic syndrome suggestive of Parkinsonism after a latency of 1–2 weeks. Moreover, pathologic analysis confirmed depletion of striatal dopamine and dopaminergic cell loss. If validated, such a model may be appropriate for testing putative neuroprotective compounds. Unfortunately, these findings have been difficult to confirm. A series of recent reports have highlighted the controversy, with a number of laboratories only partially replicating or completely failing to replicate the model (reviewed in [63^{••},64[•]–68[•]]). In light of this uncertainty, the proteasomal inhibition model, despite its early promise, requires further validation before it can be used to screen compounds for potential clinical use.

Conclusion

Angst over the disappointing results from clinical neuroprotection trials for Parkinson's disease has engendered efforts to optimize neuroprotection trial design, as well as to refine the animal models in which candidate neuroprotectants are advanced. The early use of clinical measures such as time until dopaminergic therapy or change in clinical rating scales as primary outcomes were complicated by confounding effects of symptomatic improvement. While the use of delayed start designs may help to overcome these confounders, there is nevertheless a need to develop better, more objective biomarkers of disease progression. Futility studies may prove to be helpful to exclude more efficiently compounds that are unlikely to be useful. The variability in the calibration placebo in the first two futility trials, however, raises concern that compounds may be deemed futile or not futile inappropriately. In reexamining the process by which promising neuroprotectants are identified, the validity of our toxin-based animal models has been questioned, prompting new models that more closely mimic the progressive features and pathology of the disease. Continued advances in our understanding of genetic and environmental factors contributing to Parkinson's disease will lead to more promising

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therapeutic candidates. The prospects for their translation into clinical practice will be enhanced by further improvements in animal models and clinical trial designs for Parkinson's disease.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Suchowersky O, Gronseth G, Perlmutter J, *et al.* Practice parameter: neuroprotective strategies and alternative therapies for Parkinson disease (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2006; 66:976–982.
- A systematic analysis of clinical trials for neuroprotection and alternative therapies, with recommendations for practice, concluding there are no proven effective neuroprotective therapies for Parkinson's disease.
- 2 Poewe W. The need for neuroprotective therapies in Parkinson's disease:
 - a clinical perspective. *Neurology* 2006; 66:S2–S9.
 - A review of the clinical symptomatology of Parkinson's disease, with emphasis on the unmet need for neuroprotective agents.
- 3 Tanner CM, Ottman R, Goldman SM, *et al.* Parkinson disease in twins: an etiologic study. *JAMA* 1999; 281:341–346.
- 4 Dawson TM, Dawson VL. Neuroprotective and neurorestorative strategies for Parkinson's disease. *Nat Neurosci* 2002; 5 (Suppl):1058–1061.
- 5 Fahn S, Sulzer D. Neurodegeneration and neuroprotection in Parkinson disease. *NeuroRx* 2004; 1:139–154.
- 6 Ravina BM, Fagan SC, Hart RG, *et al.* Neuroprotective agents for clinical trials in Parkinson's disease: a systematic assessment. *Neurology* 2003; 60:1234–1240.
- 7 Effect of deprenyl on the progression of disability in early Parkinson's disease. The Parkinson Study Group. *N Engl J Med* 1989; 321:1364–1371.
- 8 Tetrad JW, Langston JW. The effect of deprenyl (selegiline) on the natural history of Parkinson's disease. *Science* 1989; 245:519–522.
- 9 Palhagen S, Heinonen EH, Hagglund J, *et al.* Selegiline delays the onset of disability in de novo parkinsonian patients. Swedish Parkinson Study Group. *Neurology* 1998; 51:520–525.
- 10 Olanow CW, Hauser RA, Gauger L, *et al.* The effect of deprenyl and levodopa on the progression of Parkinson's disease. *Ann Neurol* 1995; 38:771–777.
- 11 Larsen JP, Boas J, Erdal JE. Does selegiline modify the progression of early Parkinson's disease? Results from a five-year study. The Norwegian-Danish Study Group. *Eur J Neurol* 1999; 6:539–547.
- 12 Parkinson Study Group. Effect of lazabemide on the progression of disability in early Parkinson's disease. *Ann Neurol* 1996; 40:99–107.
- 13 Parkinson Study Group. A controlled, randomized, delayed-start study of rasagiline in early Parkinson disease. *Arch Neurol* 2004; 61:561–566.
- 14 Palhagen S, Heinonen E, Hagglund J, *et al.* Selegiline slows the progression of the symptoms of Parkinson disease. *Neurology* 2006; 66:1200–1206.
- 15 Parkinson Study Group. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. *N Engl J Med* 1993; 328:176–183.
- 16 Shults CW, Oakes D, Kieburtz K, *et al.* Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline. *Arch Neurol* 2002; 59:1541–1550.
- 17 NINDS NET-PD Investigators. A randomized clinical trial of coenzyme Q10
 - and GPI-1485 in early Parkinson disease. *Neurology* 2007; 68:20–28.
 - This phase II futility trial fails to rule out coenzyme Q10 or GPI-1485 as potential neuroprotective agents, but highlights methodological issues regarding constructing futility thresholds.
- 18 Bender A, Koch W, Elstner M, *et al.* Creatine supplementation in Parkinson
 - disease: a placebo-controlled randomized pilot trial. *Neurology* 2006; 67:1262–1264.
 - The first reported pilot, double-blind placebo-controlled clinical trial for creatine demonstrates no benefit on overall UPDRS or dopamine transporter SPECT.
- 19 NINDS NET-PD Investigators. A randomized, double-blind, futility clinical trial
 - of creatine and minocycline in early Parkinson disease. *Neurology* 2006; 66:664–671.
 - This randomized futility study concludes that creatine and minocycline cannot be excluded as possible neuroprotective agents.
- 20 Parkinson Study Group. Dopamine transporter brain imaging to assess the effects of pramipexole vs levodopa on Parkinson disease progression. *JAMA* 2002; 287:1653–1661.
- 21 Whone AL, Watts RL, Stoessl AJ, *et al.* Slower progression of Parkinson's disease with ropinirole versus levodopa: The REAL-PET study. *Ann Neurol* 2003; 54:93–101.
- 22 Fahn S, Oakes D, Shoulson I, *et al.* Levodopa and the progression of Parkinson's disease. *N Engl J Med* 2004; 351:2498–2508.
- 23 Jankovic J, Hunter C. A double-blind, placebo-controlled and longitudinal study of riluzole in early Parkinson's disease. *Parkinsonism Relat Disord* 2002; 8:271–276.
- 24 Nutt JG, Burchiel KJ, Comella CL, *et al.* Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology* 2003; 60:69–73.
- 25 Lang AE, Gill S, Patel NK, *et al.* Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. *Ann Neurol* 2006; 59:459–466.
- 26 Poulter MO, Payne KB, Steiner JP. Neuroimmunophilins: a novel drug therapy for the reversal of neurodegenerative disease? *Neuroscience* 2004; 128:1–6.
- 27 Olanow CW, Schapira AH, LeWitt PA, *et al.* TCH346 as a neuroprotective
 - drug in Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol* 2006; 5:1013–1020.
 - This double-blind, randomized controlled trial demonstrates that TCH346 did not show evidence for neuroprotection. The authors present an excellent discussion of possible reasons for the discrepancy between promising preclinical data and negative clinical outcome.
- 28 Shoulson I, Parkinson Study Group PRECEPT Investigators. CEP-1347 treatment fails to favorably modify the progression of Parkinson's disease (PRECEPT) study. *Neurology* 2006; 67:185.
- 29 Hauser RA, Zesiewicz TA. Clinical trials aimed at detecting neuroprotection in
 - Parkinson's disease. *Neurology* 2006; 66:S58–S68.
 - A comprehensive review of prior clinical trials in neuroprotection, highlighting limitations of clinical and imaging outcome measures.
- 30 Ravina B, Eidelberg D, Ahlskog JE, *et al.* The role of radiotracer imaging in Parkinson disease. *Neurology* 2005; 64:208–215.
- 31 Shults CW, Flint Beal M, Song D, Fontaine D. Pilot trial of high dosages of coenzyme Q10 in patients with Parkinson's disease. *Exp Neurol* 2004; 188:491–494.
- 32 Matthews RT, Ferrante RJ, Klivenyi P, *et al.* Creatine and attenuate MPTP neurotoxicity. *Exp Neurol* 1999; 157:142–149.
- 33 Stefanis L, Burke RE, Greene LA. Apoptosis in neurodegenerative disorders. *Curr Opin Neurol* 1997; 10:299–305.
- 34 Yuan J, Yankner BA. Apoptosis in the nervous system. *Nature* 2000; 407:802–809.
- 35 Mattson MP. Apoptosis in neurodegenerative disorders. *Nat Rev Mol Cell Biol* 2000; 1:120–129.
- 36 Vila M, Przedborski S. Targeting programmed cell death in neurodegenerative diseases. *Nat Rev Neurosci* 2003; 4:365–375.
- 37 Olanow CW. Rationale for considering that propargylamines might be
 - neuroprotective in Parkinson's disease. *Neurology* 2006; 66:S69–S79.
 - This review discusses the preclinical evidence and mechanism of action for the propargylamine compounds selegiline, rasagiline, and TCH346 as putative neuroprotective agents, as well as the results of clinical trials to date.
- 38 Hara MR, Thomas B, Cascio MB, *et al.* Neuroprotection by pharmacologic blockade of the GAPDH death cascade. *Proc Natl Acad Sci U S A* 2006; 103:3887–3889.
- 39 Andringa G, Eshuis S, Perentes E, *et al.* TCH346 prevents motor symptoms and loss of striatal FDOPA uptake in bilaterally MPTP-treated primates. *Neurobiol Dis* 2003; 14:205–217.
- 40 Andringa G, van Oosten RV, Unger W, *et al.* Systemic administration of the propargylamine CGP 3466B prevents behavioural and morphological deficits in rats with 6-hydroxydopamine-induced lesions in the substantia nigra. *Eur J Neurosci* 2000; 12:3033–3043.
- 41 Silva RM, Kuan CY, Rakic P, Burke RE. Mixed lineage kinase-c-jun N-terminal kinase signaling pathway: a new therapeutic target in Parkinson's disease. *Mov Disord* 2005; 20:653–664.

- 42 Zhang GY, Zhang QG. Agents targeting c-Jun N-terminal kinase pathway as potential neuroprotectants. *Expert Opin Investig Drugs* 2005; 14:1373–1383.
- 43 Waldmeier P, Bozyczko-Coyne D, Williams M, Vaught JL. Recent clinical failures in Parkinson's disease with apoptosis inhibitors underline the need for a paradigm shift in drug discovery for neurodegenerative diseases. *Biochem Pharmacol* 2006; 72:1197–1206.
- Thorough review of the preclinical evidence for apoptosis inhibitors as neuroprotective agents, and discussion of the possible reasons for lack of effect in clinical trials.
- 44 Burke RE, Kholodilov NG. Programmed cell death: does it play a role in Parkinson's disease? *Ann Neurol* 1998; 44 (3 Suppl 1):S126–S133.
- 45 Hartmann A, Troadec JD, Hunot S, *et al.* Caspase-8 is an effector in apoptotic death of dopaminergic neurons in Parkinson's disease, but pathway inhibition results in neuronal necrosis. *J Neurosci* 2001; 21:2247–2255.
- 46 Kiebertz K. Issues in neuroprotection clinical trials in Parkinson's disease. •• *Neurology* 2006; 66:S50–S57.
- An excellent discussion of recent concepts in clinical study design.
- 47 Heikkila RE, Duvoisin RC, Finberg JP, Youdim MB. Prevention of MPTP-induced neurotoxicity by AGN-1133 and AGN-1135, selective inhibitors of monoamine oxidase-B. *Eur J Pharmacol* 1985; 116:313–317.
- 48 Parkinson Study Group. A controlled trial of rasagiline in early Parkinson disease: the TEMPO Study. *Arch Neurol* 2002; 59:1937–1943.
- 49 Rascol O, Olanow CW. ADAGIO: a prospective double-blind delayed-start study to examine potential disease-modifying effect of rasagiline in Parkinson's disease. *Mov Disord* 2006; 21 (Suppl 15):S570.
- 50 Elm JJ, Goetz CG, Ravina B, *et al.* A responsive outcome for Parkinson's disease neuroprotection futility studies. *Ann Neurol* 2005; 57:197–203.
- 51 Tilley BC, Palesch YY, Kiebertz K, *et al.* Optimizing the ongoing search for new •• treatments for Parkinson disease: using futility designs. *Neurology* 2006; 66:628–633.
- This report discusses the methodology behind futility studies, using examples from the DATATOP study to discuss the advantages and limitations of this study design.
- 52 Schwid SR, Cutter GR. Futility studies: spending a little to save a lot. • *Neurology* 2006; 66:626–627.
- Commentary on the potential benefits of futility study design.
- 53 Du Y, Ma Z, Lin S, *et al.* Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proc Natl Acad Sci U S A* 2001; 98:14669–14674.
- 54 Gold BG, Nutt JG. Neuroimmunophilin ligands in the treatment of Parkinson's disease. *Curr Opin Pharmacol* 2002; 2:82–86.
- 55 Zhang C, Steiner JP, Hamilton GS, *et al.* Regeneration of dopaminergic function in 6-hydroxydopamine-lesioned rats by neuroimmunophilin ligand treatment. *J Neurosci* 2001; 21:RC156.
- 56 Beal MF. Experimental models of Parkinson's disease. *Nat Rev Neurosci* 2001; 2:325–334.
- 57 Betarbet R, Sherer TB, Greenamyre JT. Animal models of Parkinson's disease. *Bioessays* 2002; 24:308–318.
- 58 Fornai F, Schluter OM, Lenzi P, *et al.* Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin–proteasome system and alpha-synuclein. *Proc Natl Acad Sci U S A* 2005; 102:3413–3418.
- 59 Anderson DW, Bradbury KA, Schneider JS. Neuroprotection in Parkinson • models varies with toxin administration protocol. *Eur J Neurosci* 2006; 24:3174–3182.
- This study reports that efficacy of putative neuroprotective agents in the MPTP mouse model depends on how the toxin is administered.
- 60 Bove J, Prou D, Perier C, Przedborski S. Toxin-induced models of Parkinson's disease. *NeuroRx* 2005; 2:484–494.
- 61 McNaught KS, Perl DP, Brownell AL, Olanow CW. Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. *Ann Neurol* 2004; 56:149–162.
- 62 McNaught KS, Olanow CW. Proteasome inhibitor-induced model of • Parkinson's disease. *Ann Neurol* 2006; 60:243–247.
- One in a series of articles discussing the reproducibility of proteasomal inhibition animal model of Parkinson's disease.
- 63 Beal F, Lang A. The proteasomal inhibition model of Parkinson's disease: •• 'Boon or bust'? *Ann Neurol* 2006; 60:158–161.
- This review summarizes the data presented in references [57,59*,60,61,62*,63**] supporting and refuting the use of proteasome inhibitors to induce Parkinsonism in rodents.
- 64 Zeng BY, Bukhatwa S, Hikima A, *et al.* Reproducible nigral cell loss after • systemic proteasomal inhibitor administration to rats. *Ann Neurol* 2006; 60:248–252.
- One in a series of articles discussing the reproducibility of the proteasomal inhibition animal model of Parkinson's disease.
- 65 Schapira AH, Cleeter MW, Muddle JR, *et al.* Proteasomal inhibition causes • loss of nigral tyrosine hydroxylase neurons. *Ann Neurol* 2006; 60:253–255.
- One in a series of articles discussing the reproducibility of the proteasomal inhibition animal model of Parkinson's disease.
- 66 Bove J, Zhou C, Jackson-Lewis V, *et al.* Proteasome inhibition and Parkinson's • disease modeling. *Ann Neurol* 2006; 60:260–264.
- One in a series of articles discussing the reproducibility of the proteasomal inhibition animal model of Parkinson's disease.
- 67 Manning-Bog AB, Reaney SH, Chou VP, *et al.* Lack of nigrostriatal pathology • in a rat model of proteasome inhibition. *Ann Neurol* 2006; 60:256–260.
- One in a series of articles discussing the reproducibility of the proteasomal inhibition animal model of Parkinson's disease.
- 68 Kordower JH, Kanaan NM, Chu Y, *et al.* Failure of proteasome inhibitor • administration to provide a model of Parkinson's disease in rats and monkeys. *Ann Neurol* 2006; 60:264–268.
- One in a series of articles discussing the reproducibility of the proteasomal inhibition animal model of Parkinson's disease.

Serum urate as a predictor of clinical and radiographic progression in Parkinson's disease

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ABSTRACT

Context Prospective epidemiological studies consistently indicate that Parkinson's disease (PD) risk declines with increasing serum urate.

Objective To determine whether serum urate, a purine metabolite and potent antioxidant, predicts prognosis in PD.

Design, Setting, and Participants Prospective study among 804 subjects with early PD enrolled in the PRECEPT study, a clinical trial of the neuroprotectant potential of CEP-1347, conducted between April 2002 and August 2005 (average follow-up time 21.4 months).

Main Outcome Measures The primary study endpoint was progression to clinical disability sufficient to warrant dopaminergic therapy. Cox proportional hazards models were used to estimate the hazard ratio (HR) of reaching endpoint according to quintiles of baseline serum urate, adjusting for gender, age and other potential covariates. Change in striatal uptake of [123 I] β -CIT, a marker for the presynaptic dopamine transporter, was assessed with linear regression for a subset of 399 subjects.

Results The adjusted HR of reaching endpoint declined with increasing baseline concentrations of urate; subjects in the top quintile reached the endpoint at only half the rate of subjects in the bottom quintile (HR=0.51; 95% CI: 0.37 to 0.72; p=0.0002). This association was markedly stronger in men (HR=0.39; 95% CI: 0.26 to 0.60; p<0.0001) than in women (HR=0.77; 95% CI: 0.39 to 1.50; p=0.4). The percent loss in striatal [123 I] β -CIT uptake also improved with increasing serum urate concentrations (overall p for trend=0.002; men, p=0.0008; women, p=0.4).

Conclusion These findings identify serum urate as the first molecular factor directly linked to the progression of typical PD and suggest that targeting urate or its determinants could be an effective disease modifying therapy in PD.

As a consequence of mutations in the *urate oxidase* gene early in primate evolution,¹ urate in humans circulates at high concentrations near the limits of its solubility and constitutes the main end product of purine metabolism. Urate has an anti-oxidant efficacy comparable to that of ascorbate, and thus its high level serves as one of our major defenses against oxidative damage caused by reactive nitrogen and oxygen species.² Because oxidative stress may contribute to the loss of dopaminergic neurons in the substantia nigra of individuals with Parkinson's disease (PD)³ and to the pathophysiology of other neurodegenerative diseases,⁴ blood urate could be an important determinant of disease susceptibility and progression.

Supporting this notion, the results of prospective epidemiological studies consistently indicate that among healthy people the risk of PD declines with increasing uricemia.^{5,6,7} Whether uricemia also predicts a better prognosis in established PD had not, however, been investigated. To address this question efficiently we sought completed, rigorously conducted clinical studies of PD progression in which prospectively determined (baseline) levels of urate are available. We identified a large randomized clinical trial entitled, "Parkinson Research Examination of CEP-1347 Trial" (PRECEPT), which was originally designed to investigate a candidate neuroprotectant in PD using clinical and imaging assessments of neurodegeneration,⁸ as an ideal opportunity to evaluate the potential association between serum urate and subsequent rates of PD progression.

METHODS

Study design

We conducted a longitudinal cohort investigation among participants in the PRECEPT study, a two-year double-blind, randomized trial of oral CEP-1347, an antiapoptotic mixed lineage kinase inhibitor that had been found to be neuroprotective in animal models of PD.⁸ The PRECEPT study was designed to determine whether this drug could slow the progression of early PD. It was carried out by the Parkinson Study Group, and sponsored by Cephalon, Inc. and H. Lundbeck A/S. The participants (n=806) were enrolled between April 2002 and April 2004 at 65 sites across the United States and Canada. All participating sites obtained approval of the protocol by their institutional review boards, and all subjects gave written consent for study participation.

Study population

Subjects to be enrolled in the study had to have early PD (modified Hoehn and Yahr stage of ≤ 2.5 with two of the cardinal signs: resting tremor, bradykinesia or rigidity), not requiring the use of dopaminergic therapy, and age at diagnosis ≥ 30 years.⁸ Exclusion criteria included atypical parkinsonism, a diagnosis of PD ≥ 5 years duration, a tremor score ≥ 3 , a Mini-Mental Status Exam score ≤ 26 , a Beck Depression Inventory score ≥ 15 , the use of symptomatic therapy within six months prior to randomization, or an expectation that dopaminergic therapy would be required within three months of study

enrollment. Subjects were randomly assigned to receive placebo or CEP-1347 10 mg, 25 mg, or 50 mg twice daily.

Serum urate and covariates

Serum urate was measured at screening as well as the subsequent baseline visit (on average 4 weeks apart) as one component of the routine safety monitoring performed. The correlation between the screening and baseline serum urate was high ($r=0.88$; $p<0.0001$), and only baseline values, which were available for 99.8% of patients enrolled in the trial, have been used in the present analyses. Serum urate levels were determined using an enzymatic assay on non-fasting blood, performed at a central commercial clinical laboratory (Covance, Indianapolis, IN). Information on past medical history and regular use of medications was collected at the screening visit.

Clinical evaluation

Following the initial screening and baseline visits, subjects were seen one month after starting the study drug, and then every three months until 24 months had elapsed. At each visit the site investigator conducted a clinical assessment, and evaluated the subject for disability sufficient to require dopaminergic therapy, the primary endpoint for the study.

A follow-up visit was performed one month after discontinuing the study medication. Of the 806 randomized patients, 95 (11.8%) were censored, 48 because of withdrawal and 47 because they requested dopaminergic treatment before this was considered required by the investigators. The average follow-up time was 21.4 months.⁸

Outcome

The primary endpoint was time to disability requiring dopaminergic therapy,^{9, 8} determined by individual investigators masked to treatment assignment. Secondary endpoints included changes in the Unified PD Rating Scale (UPDRS) (sum of the motor, cognitive, and activity of daily living subscales) and β -CIT SPECT imaging of ligand binding to striatal dopamine transporter, a marker for nigrostriatal dopaminergic nerve terminals. Because the UPDRS is modified by the dopaminergic treatment instituted at endpoint, the annualized rate of change in UPDRS was determined based on change from baseline to endpoint for each subject, and was calculated as: $[(\text{total UPDRS at the last assessment before initiation of dopaminergic treatment} - \text{total UPDRS at baseline}) / \text{number of days between the two assessments}) \times (365 \text{ days/year})$.

Neuroimaging substudy

Single-photon emission computed-tomography (SPECT) of iodine-123-labeled 2- β -carboxymethoxy-3- β -(4-iodophenyl)tropane ($[^{123}\text{I}]\beta$ -CIT) uptake was used at baseline to measure striatal dopamine-transporter density among all subjects in the trial; imaging was carried out at the Institute for Neurodegenerative Disorders in New Haven, Connecticut with methods as described previously.¹⁰ All subjects were invited to repeat the SPECT at the end of the follow-up. The 399 subjects with repeated SPECT imaging completed as of May 2005 and with baseline serum urate were included in a subanalyses on the relation between baseline serum urate and percent change in the ratio of the specific striatal $[^{123}\text{I}]\beta$ -CIT uptake to the non-displaceable striatal $[^{123}\text{I}]\beta$ -CIT uptake between the two images. Mean interval between the two SPECT scans was 22 months.

Statistical analysis

Cox proportional hazards models were used to estimate the hazard ratios of reaching the endpoint according to quintiles of baseline serum urate, adjusting for gender and age (5-year groups). Initial analyses were conducted using quintiles based on the combined urate distribution in men and women ("common quintiles"). An important advantage of using common quintiles is that the hazard ratios in men and women estimate the effects of similar levels of serum urate. However, because of the expected higher level of urate in men, this categorization resulted in a markedly skewed distribution within gender, with most men in the top quintiles and most women in the bottom quintiles of serum urate, and thus in a loss of power of analyses within gender. These analyses were therefore complemented by estimating hazard ratios for gender-specific quintiles. In these analyses the advantage of a more balanced distribution of subjects across quintiles is in part offset by the lack of comparability of the hazard ratios; for example, in men the cut-offs for lowest and highest quintiles are < 4.9 and > 7.0 mg/dL, versus < 3.7 and > 5.6 mg/dL in women. Tests for trend were conducted by including serum urate as a continuous variable in the proportional hazard models (Wald test). Potential confounding was assessed by adjusting the regression analyses for cigarette smoking (never, past, or current), body mass index (quintiles), serum cholesterol (continuous), and use of antihypertensive drugs or non-steroidal antiinflammatory drugs (use versus no-use). Because the adjusted results were not materially different from the unadjusted, and none of these covariates was significantly related to PD progression, only the age- and gender-adjusted results have been included in this report. Possible interactions between serum urate and age, gender, and treatment group were explored by including in the proportional hazard model the cross-product of serum urate as a continuous variable with the corresponding covariates (age: continuous in years; gender: 0,1 ; treatment: 0, 1, 2, 3, for placebo and each of the CEP-1347 doses). None of the interaction terms was significant, and only results not including these terms are reported. There was no significant deviation from the proportional hazard assumption, tested by adding the cross product of urate (continuous) with time of follow-up (0 for first year, 1 thereafter) to the Cox model.

The relation between serum urate and rate of change in UPDRS or percent change in striatal [123 I] β -CIT uptake were assessed by linear regression. For each of these outcomes, we fitted regression models including age, gender (for analyses including men and women combined), and either common quintiles of serum urate or gender-specific quintiles, as outlined above. Because of the skewed distribution of UPDRS rates, analyses for this outcome were also conducted using Spearman correlation. All the p values presented are for 2-tailed tests with levels < 0.05 defined as significant.

RESULTS

Serum urate at baseline was available for 804 (517 men and 287 women) of the 806 subjects enrolled in the trial. Selected characteristics of these subjects are shown in Table 1. As expected, serum urate concentrations were positively correlated with male gender,

body mass index, use of thiazide diuretics, and history of gout and hypertension (Table 1).

Overall, 493 (61%) of participants reached the endpoint of disability sufficient to require dopaminergic therapy during follow-up. The hazard ratio of reaching the endpoint declined with increasing concentrations of serum urate; subjects in the top common quintile reached endpoint at approximately half the rate of subjects in the bottom quintile (HR = 0.51; 95% CI: 0.37 to 0.72; $p < 0.0001$) (Table 2). This association was markedly stronger in men than in women, although a test for interaction of urate with gender was not significant (Table 2). Results of analyses based on gender-specific quintiles were similar, and are summarized in Kaplan-Meier curves (Figure 1).

The rate of change in UPDRS (points per year) in men and women combined was 16.9 among patients in the lowest quintile of baseline urate, and 14.3 among those in the highest quintile (p for trend = 0.09). Among men, there was a modest but significant inverse association between baseline serum urate and rate of UPDRS change (Spearman correlation coefficient = -0.10; $p = 0.02$). A significantly lower rate of change in UPDRS was observed among patients in the highest as compared with those in the lowest gender-specific quintile of serum urate (adjusted difference = 7.0; $p = 0.02$). In contrast, no significant association was found in women (Spearman $r = -0.03$, $p = 0.5$).

The percent change in striatal [^{123}I] β -CIT uptake also declined with increasing serum urate concentrations (p for trend = 0.002), although the trend was largely driven by a lower percent change among subjects in the top quintile of serum urate, with little or no differences between quintiles 1 through 4 (Figure 2). Because there were only 4 women in the top quintile of serum urate when the cut-offs for quintiles were generated from men and women combined, stratified analyses were only conducted using gender-specific quintiles. As in the endpoint analyses, a significant association was only seen in men (Figure 2).

COMMENT

In this large prospective investigation among subjects in the early stages of PD enrolled in a randomized clinical trial, we found that the rate of progression to the primary clinical endpoint declined with increasing levels of baseline serum urate. There was a clear dose-response relationship with a 35% reduction in rate of progression among patients in the fourth quintile of serum urate and a 49% reduction among those in the highest quintile, as compared with those in the lowest quintile. These associations were highly significant, and corroborated by the finding that patients with higher urate also had a lower percent loss of striatal [^{123}I] β -CIT uptake during the follow-up.

Strengths of this study include the longitudinal design, the measurement of serum urate at baseline and before starting any anti-parkinsonian treatment, the large number of participants, and the rigorous clinical assessment of all patients. We specifically examined the relation between serum urate and PD progression because of the strong *a priori* evidence that individuals with high levels of serum urate have a markedly reduced

risk of developing PD.⁵⁻⁷ The convergence between the results of previous epidemiological studies and those of the present investigation is striking. Combined, these results support the continuity of the neurodegenerative process before and after the onset of the first motor symptoms that lead to the diagnosis of PD, and imply that either higher serum urate itself is neuroprotective, or it serves as an indirect marker of protection of the dopaminergic neurons that are lost in PD.

The inverse association between uricemia and PD progression could be explained if both were affected by a common factor, or, in epidemiological lexicon, a confounder. In subjects without PD, the strongest correlates of serum urate are male gender, obesity, and arterial hypertension.¹¹ Further, use of thiazide diuretics is known to increase urate levels.¹² These correlations were also found among participants in our study, suggesting that the main determinants of serum urate are the same in individual with or without PD. However, the relation between serum urate and PD progression in our study was independent from these factors. Also, adjustment for cigarette smoking and use of non-steroidal anti-inflammatory drugs, which have been related to PD risk, did not appreciably change the results. Genetic factors could also affect both serum urate and PD progression, and thus act as confounders. Heritability of serum urate is estimated to range from 25% to 70%¹³, and several genetic mutations that affect uricemia have been identified.¹⁴⁻¹⁶ Known mutations with marked effects on uricemia, however, are rare and seem unlikely to fully explain the strong inverse associations between uricemia and both PD risk and PD progression. Finally, dietary factors should also be considered. High dairy consumption has been associated with an increased risk of PD¹⁷⁻¹⁹ and with decreased serum urate²⁰, but the latter association is weak and unlikely to account for much variation in urate levels. On the other hand, high alcohol consumption increases serum urate,²¹ but in longitudinal studies alcohol consumption was not consistently related to PD risk.²² Purine²⁰ and fructose²³ intakes also increase serum urate, but these effects are modest and there is no evidence that these nutrients would affect PD risk or progression independently from their effects on uricemia. Overall, it seems therefore unlikely that the inverse relation between uricemia and PD progression is due to confounding by known factors. As in all observational studies, however, a role for unknown factors cannot be excluded.

Although several clinical features of PD (e.g., prominent asymmetry, rest tremor predominance, absence of early cognitive or gait dysfunction) have been identified previously as predictors of a slower rate of clinical progression^{24, 25} these are complex behavioral characteristics of the disease and are thus likely to result from, rather than influence pathogenic mechanisms. By contrast, as an antioxidant with peroxynitrite scavenging properties, urate is well positioned to serve as a neuroprotectant against the underlying neurodegeneration of PD. Considerable evidence from genetic as well as idiopathic forms of PD has implicated oxidative and nitrative stress as central pathogenic mechanisms.^{26, 27} Urate at physiological concentrations is as effective an antioxidant as ascorbate.² It also stabilizes ascorbate,²⁸ possibly by forming complexes with iron ions,²⁹ and scavenges nitrogen radicals.^{30, 31} Further, administration of urate reduced the exacerbation of the oxidative stress and mitochondrial dysfunction in human dopaminergic cells exposed to the pesticide rotenone or to iron ions.³²

Alternatively, the predictive association between urate and PD progression could reflect a neuroprotective effect of a urate precursor, rather than urate itself. For example, adenosine and its deaminated metabolite inosine (which is in turn deribosylated and oxidized to urate) both modulate neuronal death on their own. Adenosine can have either neuroprotective or neurotoxic effects on dopaminergic neurons via adenosine A₁ and A_{2A} receptors, respectively.^{33, 34} Inosine has also shown potential as a neuroprotectant in models of stroke and multiple sclerosis.^{35, 36} Whether urate, its metabolic precursors or other determinants modulate neurodegeneration in PD, their potential is supported by lower levels of urate in cerebrospinal fluid³⁷ and post-mortem substantia nigra³⁸ of patients with PD.

Whereas the concentration-dependent inverse relationship was robust and highly significant statistically in men, it appeared as a weak non-significant trend amongst women. This difference between men and women (also noted for the association between urate and [¹²³I]β-CIT uptake neuroimaging) could result in part from a biological effect of gender on urate mechanisms in PD.³⁹ Alternatively, it may reflect the substantially lower average urate concentrations in women, who comprise only 16% of the subjects in the two uppermost quintiles in which the substantially slower rates of disease progression were observed.

That urate and its metabolic pathway are particularly amenable to existing pharmacological and dietary manipulations enhances the potential therapeutic significance of the present findings. A purine-rich diet can elevate serum urate, and the purine supplement inosine, used as a potential therapy for multiple sclerosis in a phase II randomized clinical trial, markedly and chronically raised urate concentrations without inducing gout or other adverse effects.^{40 41} It is also well known that thiazide diuretics even at low doses elevate urate by reducing its renal clearance.¹² Individuals with higher serum urate, however, have an increased risk of hypertension, coronary heart disease, and stroke.^{5, 42} Although these associations may in part be confounded by obesity and other risk factors,^{43 44} a long-term neuroprotective effect of urate or its precursors would have to be weighed against potential adverse cardiovascular effects.

It should be noted that measurement of urate on its own in newly diagnosed PD patients as an indicator of an individual patient's future rate of progression is likely to be of modest clinical utility.⁴⁵ On the other hand, urate testing may aid the rational design of neuroprotective trials in PD, particularly those targeting mechanisms (antioxidant or purinergic) that are potentially shared with urate. For example, coenzyme Q₁₀, creatine and rasagiline – potential neuroprotectants targeting oxidative stress pathways in planned neuroprotection trials – might be most effective in PD patients whose endogenous antioxidant pool (including urate) is lowest at baseline, and thus controlling for an interaction with or stratifying by urate levels at baseline may improve the power of such trials.

Of note, the present discovery of a urate link to PD progression was achieved through additional analyses of a rigorously conducted clinical trial whose database was made available to test unforeseen hypotheses upon conclusion of the primary investigation.⁸ The findings thus reflect a broader opportunity to retrospectively explore a growing repository of high quality data from neuroprotection trials for PD, Alzheimer's disease and other progressive degenerative disorders.

Author contributions: Dr. Alberto Ascherio had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design, drafting of manuscript, supervision: Schwarzschild and Ascherio.

Data analysis: Schwarzschild, Ascherio, Watts, Oakes.

Obtaining funding: Schwarzschild and Ascherio.

Acquisition and interpretation of data, and critical revision of manuscript: Schwarzschild, Schwid, Marek, Watts, Lang, Oakes, Shoulson and Ascherio

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References

1. Oda M, Satta Y, Takenaka O, Takahata N. Loss of urate oxidase activity in hominoids and its evolutionary implications. *Mol Biol Evol.* May 2002;19(5):640-653.
2. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci U S A.* 1981;78(11):6858-6862.
3. Jenner P. Oxidative stress in Parkinson's disease. *Ann Neurol.* 2003;53 Suppl 3:S26-36; discussion S36-28.
4. Beal MF. Mitochondria take center stage in aging and neurodegeneration. *Ann Neurol.* Oct 2005;58(4):495-505.
5. Davis JW, Grandinetti A, Waslien CI, Ross GW, White LR, Morens DM. Observations on serum uric acid and the risk of idiopathic Parkinson's disease. *Am J Epidemiol.* 1996;144:480-484.
6. de Lau LM, Koudstaal PJ, Hofman A, Breteler MM. Serum uric acid levels and the risk of Parkinson disease. *Ann Neurol.* Oct 20 2005;58(5):797-800.
7. Weisskopf MG, O'Reilly E, Chen H, Schwarzschild MA, Ascherio A. Plasma urate and risk of Parkinson's disease. *Am J Epidemiol.* 2007;[In Press].
8. The Parkinson Study Group PRECEPT Investigators. The mixed lineage kinase inhibitor CEP-1347 fails to delay disability in early Parkinson's disease. *Neurology.* 2007.
9. Parkinson Study Group. DATATOP: a multicenter controlled clinical trial in early Parkinson's disease. *Arch Neurol.* Oct 1989;46(10):1052-1060.
10. Marek K, Innis R, van Dyck C, et al. [123I]beta-CIT SPECT imaging assessment of the rate of Parkinson's disease progression. *Neurology.* Dec 11 2001;57(11):2089-2094.
11. Roubenoff R, Klag MJ, Mead LA, Liang KY, Seidler AJ, Hochberg MC. Incidence and risk factors for gout in white men. *JAMA.* Dec 4 1991;266(21):3004-3007.
12. Reyes AJ. Cardiovascular drugs and serum uric acid. *Cardiovasc Drugs Ther.* Sep-Nov 2003;17(5-6):397-414.
13. Yang Q, Guo CY, Cupples LA, Levy D, Wilson PW, Fox CS. Genome-wide search for genes affecting serum uric acid levels: the Framingham Heart Study. *Metabolism.* Nov 2005;54(11):1435-1441.
14. Enomoto A, Kimura H, Chairoungdua A, et al. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature.* May 23 2002;417(6887):447-452.
15. Graessler J, Graessler A, Unger S, et al. Association of the human urate transporter 1 with reduced renal uric acid excretion and hyperuricemia in a German Caucasian population. *Arthritis Rheum.* Jan 2006;54(1):292-300.
16. Berry CE, Hare JM. Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *J Physiol.* Mar 16 2004;555(Pt 3):589-606.
17. Chen H, Zhang SM, Hernan MA, Willett WC, Ascherio A. Diet and Parkinson's disease: A potential role of dairy products in men. *Ann Neurol.* Dec 2002;52(6):793-801.

18. Park M, Ross GW, Petrovitch H, et al. Consumption of milk and calcium in midlife and the future risk of Parkinson disease. *Neurology*. Mar 22 2005;64(6):1047-1051.
19. Chen H, O'Reilly E, McCullough ML, et al. Consumption of dairy products and risk of Parkinson's disease. *Am J Epidemiol*. 2007;165:998-1006.
20. Choi HK, Liu S, Curhan G. Intake of purine-rich foods, protein, and dairy products and relationship to serum levels of uric acid: the Third National Health and Nutrition Examination Survey. *Arthritis Rheum*. Jan 2005;52(1):283-289.
21. Yamamoto T, Moriwaki Y, Takahashi S. Effect of ethanol on metabolism of purine bases (hypoxanthine, xanthine, and uric acid). *Clin Chim Acta*. Jun 2005;356(1-2):35-57.
22. Hernán MA, Chen H, Schwarzschild MA, Ascherio A. Alcohol consumption and the incidence of Parkinson's disease. *Ann Neurol*. 2003;54(2):170-175.
23. Mayes PA. Intermediary metabolism of fructose. *Am J Clin Nutr*. Nov 1993;58(5 Suppl):754S-765S.
24. Marras C, Rochon P, Lang AE. Predicting motor decline and disability in Parkinson disease: a systematic review. *Arch Neurol*. Nov 2002;59(11):1724-1728.
25. Gasparoli E, Delibori D, Polesello G, et al. Clinical predictors in Parkinson's disease. *Neurol Sci*. Sep 2002;23 Suppl 2:S77-78.
26. Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci*. 2005;28:57-87.
27. Jenner P, Olanow CW. The pathogenesis of cell death in Parkinson's disease. *Neurology*. May 23 2006;66(10 Suppl 4):S24-36.
28. Stocker R, Frei B. Endogenous antioxidant defences in human blood plasma. In: Sies H, ed. *Oxidative Stress: Oxidants and Antioxidants*. San Diego: Academic Press; 1991:213-243.
29. Davies KJ, Sevanian A, Muakkassah-Kelly SF, Hochstein P. Uric acid-iron ion complexes. A new aspect of the antioxidant functions of uric acid. *Biochem J*. May 1 1986;235(3):747-754.
30. Squadrito GL, Cueto R, Splenser AE, et al. Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid. *Arch Biochem Biophys*. Apr 15 2000;376(2):333-337.
31. Whiteman M, Ketsawatsakul U, Halliwell B. A reassessment of the peroxynitrite scavenging activity of uric acid. *Ann N Y Acad Sci*. May 2002;962:242-259.
32. Duan W, Ladenheim B, Cutler RG, Kruman, II, Cadet JL, Mattson MP. Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. *J Neurochem*. 2002;80(1):101-110.
33. Delle Donne KT, Sonsalla PK. Protection against methamphetamine-induced neurotoxicity to neostriatal dopaminergic neurons by adenosine receptor activation. *J Pharmacol Exp Ther*. Dec 1994;271(3):1320-1326.
34. Xu K, Bastia E, Schwarzschild M. Therapeutic potential of adenosine A(2A) receptor antagonists in Parkinson's disease. *Pharmacol Ther*. Mar 2005;105(3):267-310.
35. Scott GS, Spitsin SV, Kean RB, Mikheeva T, Koprowski H, Hooper DC. Therapeutic intervention in experimental allergic encephalomyelitis by

- administration of uric acid precursors. *Proc Natl Acad Sci U S A*. Dec 10 2002;99(25):16303-16308.
36. Shen H, Chen GJ, Harvey BK, Bickford PC, Wang Y. Inosine reduces ischemic brain injury in rats. *Stroke*. Mar 2005;36(3):654-659.
 37. Tohgi H, Abe T, Takahashi S, Kikuchi T. The urate and xanthine concentrations in the cerebrospinal fluid in patients with vascular dementia of the Binswanger type, Alzheimer type dementia, and Parkinson's disease. *J Neural Transm Park Dis Dement Sect*. 1993;6(2):119-126.
 38. Church WH, Ward VL. Uric acid is reduced in the substantia nigra in Parkinson's disease: effect on dopamine oxidation. *Brain Res Bull*. 1994;33(4):419-425.
 39. Nilsen J, Brinton RD. Mitochondria as therapeutic targets of estrogen action in the central nervous system. *Curr Drug Targets CNS Neurol Disord*. Aug 2004;3(4):297-313.
 40. Spitsin S, Hooper DC, Leist T, Streletz LJ, Mikheeva T, Koprowski H. Inactivation of peroxynitrite in multiple sclerosis patients after oral administration of inosine may suggest possible approaches to therapy of the disease. *Mult Scler*. Oct 2001;7(5):313-319.
 41. Koprowski H, Spitsin SV, Hooper DC. Prospects for the treatment of multiple sclerosis by raising serum levels of uric acid, a scavenger of peroxynitrite. *Ann Neurol*. Jan 2001;49(1):139.
 42. Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. Uric Acid Is a Risk Factor for Myocardial Infarction and Stroke. The Rotterdam Study. *Stroke*. Jun 2006;37(6):1503-1507.
 43. Wheeler JG, Juzwishin KD, Eiriksdottir G, Gudnason V, Danesh J. Serum uric acid and coronary heart disease in 9,458 incident cases and 155,084 controls: prospective study and meta-analysis. *PLoS Med*. Mar 2005;2(3):e76.
 44. Forman JP, Choi H, Curhan GC. Plasma uric acid level and risk for incident hypertension among men. *J Am Soc Nephrol*. Jan 2007;18(1):287-292.
 45. Ware JH. The limitations of risk factors as prognostic tools. *N Engl J Med*. Dec 21 2006;355(25):2615-2617.

APPENDIX

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Table 1. Characteristics of study participants according to quintiles of baseline serum urate (n=804).

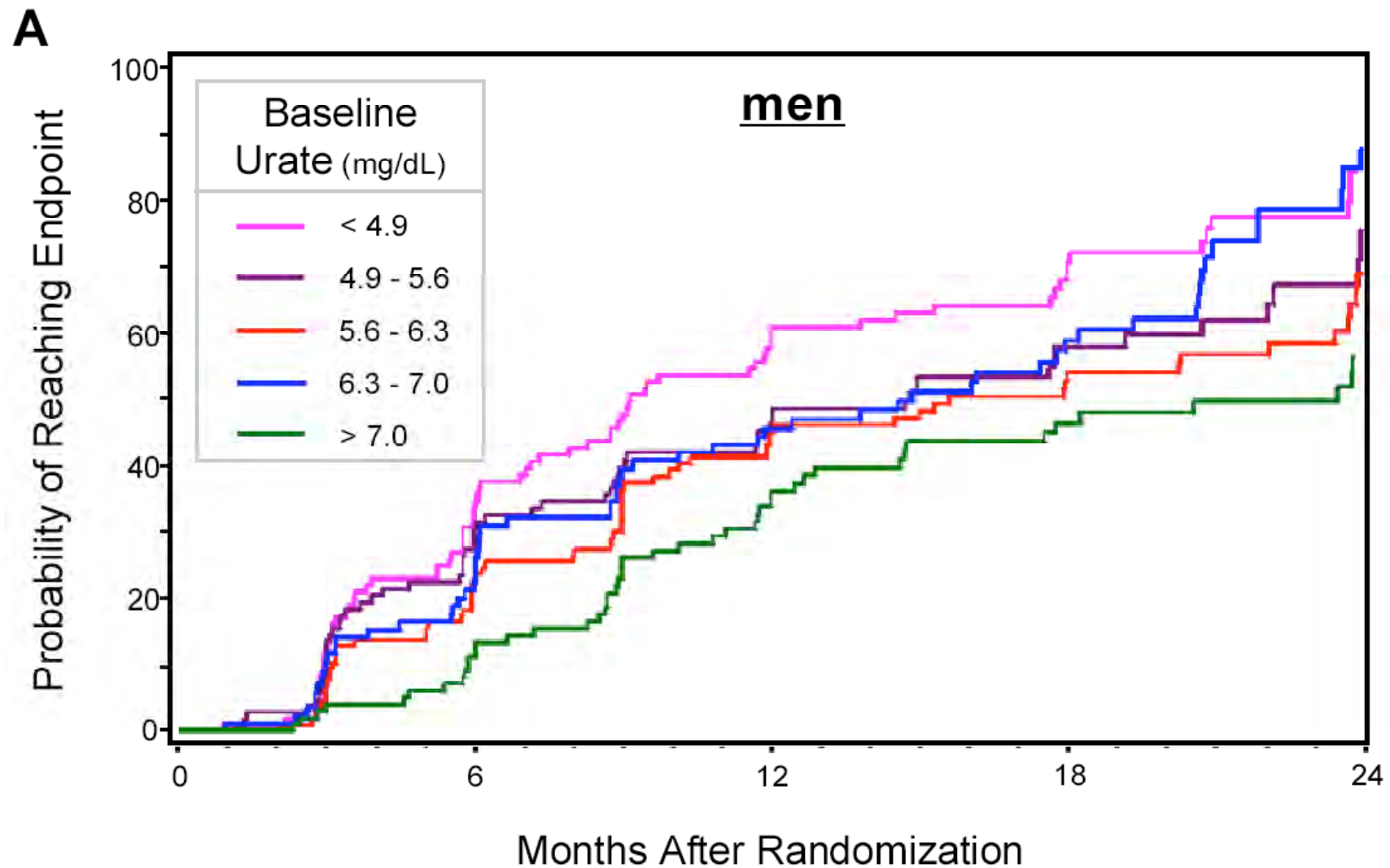
	<u>Quintile of baseline serum urate</u>					
	1	2	3	4	5	All
Serum urate (mg/dL)	<4.3	4.3-<5.1	5.1-<5.8	5.8-<6.7	≥6.7	
N. of patients	177	157	147	169	154	804
Female %	74.6	44.6	25.2	15.4	14.3	35.7
Age (median)	57	60	59	61	60	59
BMI	25.0	26.2	27.8	28.7	29.9	27.5
Current smokers %	6.2	5.1	14.3	5.3	5.2	7.1
Gout %	0	0.6	0.7	1.2	5.2	1.5
Hypertension (% use of antihypertensive drugs)	19.8	26.8	36.1	37.3	57.1	35.0
Thiazides %	5.7	6.4	10.2	14.2	16.2	10.4
NSAIDs use %	27.7	29.3	29.9	20.7	30.5	27.5
Endpoint: Yes %	67.8	61.8	64.0	59.2	53.3	61.3
UPDRS rate of change during follow-up (mean)	16.9	17.0	14.8	14.1	14.3	14.9
% change in striatal β-CIT uptake (mean)	- 10	-9	-10	-8	-4	-8

Table 2. Hazard ratios (HR)[†] for reaching the endpoint according to common quintiles of baseline serum urate.

Serum urate quintile	Median serum urate (mg/dL)	All (n=804)		Men (n=517)			Women (n=287)		
		HR [†] (95% CI)	p value	n	HR [‡] (95% CI)	p value	n	HR [‡] (95% CI)	p value
1	3.8	1.00 (Ref)	-	45	1.00 (Ref)	-	132	1.00 (Ref)	-
2	4.8	0.80 (0.60-1.07)	0.12	87	0.61 (0.40-0.94)	0.03	70	0.93 (0.63-1.37)	0.70
3	5.5	0.85 (0.63-1.15)	0.29	110	0.66 (0.44-1.00)	0.05	37	1.00 (0.61-1.64)	0.99
4	6.3	0.65 (0.47-0.88)	0.006	143	0.51 (0.34-0.76)	0.001	26	0.76 (0.41-1.39)	0.37
5	7.5	0.51 (0.37-0.72)	<0.0001	132	0.39 (0.26-0.60)	<0.0001	22	0.77 (0.39-1.50)	0.44
p, for trend			0.0002			<0.0001			0.33
p, for gender-urate interaction			0.15						

[†]Adjusted for age and gender; [‡] adjusted for age only.

Figure 1. Kaplan-Meier estimates of the cumulative probability of reaching the end point by 24 months of follow-up, according to gender-specific quintiles of baseline serum urate. A. Men; B. Women. Log-rank tests: $p=0.0011$ in men, $p=0.47$ in women. At 24 months sample size was 46 in men and 21 in women.



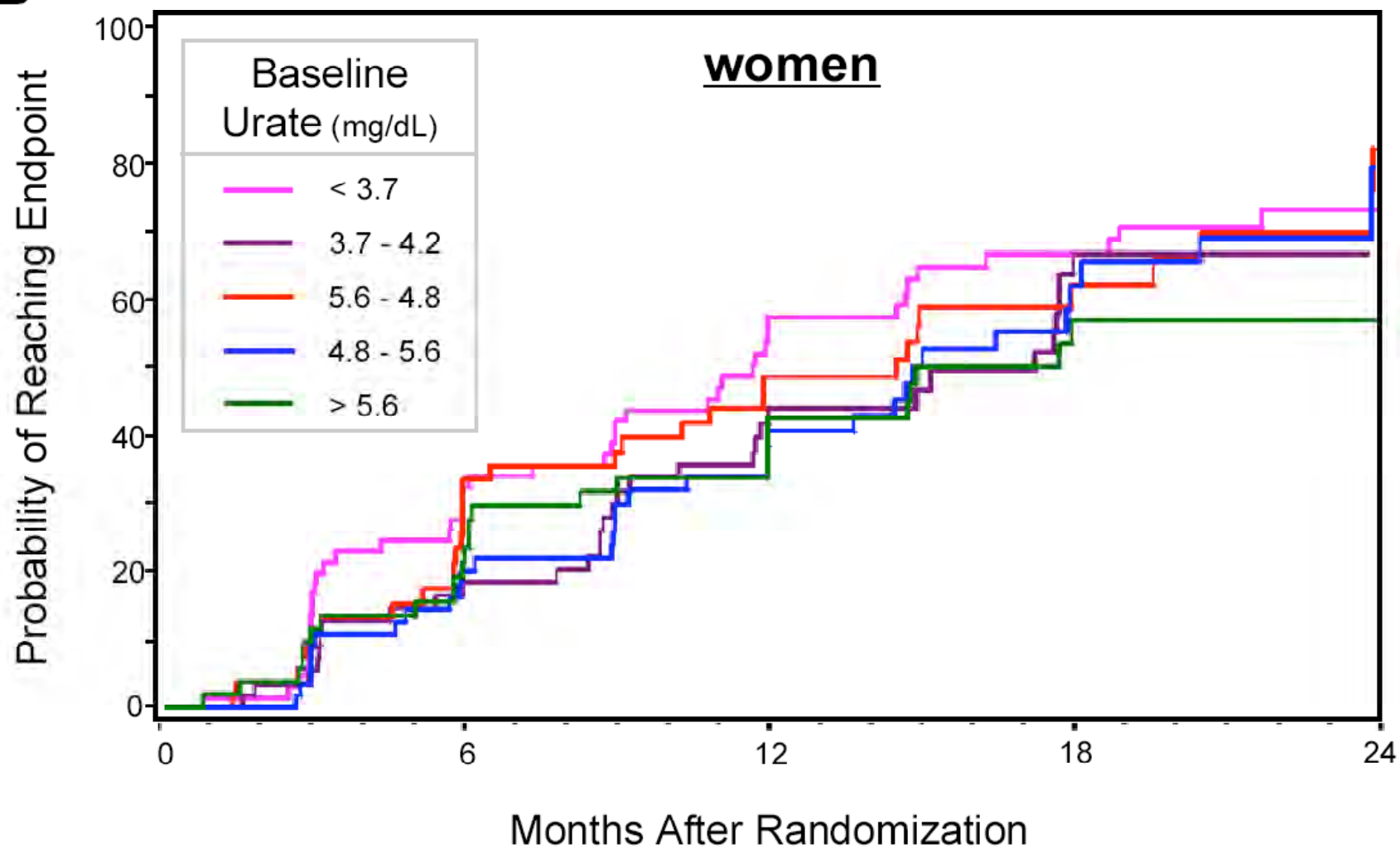
B

Figure 2. Age-adjusted percent change in striatal ^{123}I -beta-CIT uptake according to overall and gender-specific quintiles of baseline serum urate. Median serum urate by quintiles (1 to 5) mg/dL: All: 3.8, 4.8, 5.5, 6.3, 7.5 ; Men: 4.4, 5.3, 6.0, 6.6, 7.8; Women: 3.1, 4.0, 4.5, 5.2, 6.6. * $p < 0.05$; ** $p < 0.001$ compared to corresponding quintile 1. P for linear trend: all, $p = 0.002$; men, $p = 0.0008$; women, $p = 0.4$.

